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Time, Trials, and Extinction

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Abstract

Four experiments investigated the effect of number of trials and total duration of non-reinforced exposure to the conditioned stimulus (CS) on extinction of Pavlovian conditioning. Rats were first trained in a magazine approach paradigm with multiple CSs, each paired with the unconditioned stimulus (US) on a variable CS-US interval. During subsequent extinction, CSs would differ in the number and length of their extinction trials but would be matched for the total duration of exposure (e.g., one CS would have 20 trials per session with a mean length of 5 s, another CS would have 5 trials per session with a mean length of 20 s). In each case, extinction proceeded more quickly for the CS given more trials per session. Indeed, there was no difference in rate of extinction between CSs that were matched on number of trials but differed on the duration of each trial, indicating that duration of exposure has no effect on extinction. We discuss the implications of these findings for trial-based and time-based theories of conditioning.

Key words: Rat; delay conditioning; magazine approach; variable interval.

Animals respond to a conditioned stimulus (CS) when they learn that it signals a biologically significant unconditioned stimulus (US), and they stop responding to the CS if they learn that it ceases to signal the US. Modern accounts of extinction argue that the cessation of responding is driven by a mismatch between an expectancy that the US will occur, based on prior learning about the CS-US association, and absence of the US (Mackintosh, 1974). According to a broad class of models, often termed “associative models”, animals first learn an association between the CS and US during conditioning; this association is progressively weakened, or an inhibitory association is learned, when the CS is presented without the US during extinction (e.g., Pearce & Hall, 1980; Rescorla & Wagner, 1972; Wagner, 1981). Extinction follows a simple learning rule that progressively reduces the strength of the CS-US association (or strengthens an inhibitory association) as a proportion of the residual associative strength that results from previous learning about the CS and US. Associative models can be contrasted with information-based models of learning that assume that animals specifically learn about the time or rate at which the US occurs during the CS (Gallistel & Balsam, 2014; Gallistel & Gibbon, 2000). These models also argue that extinction is driven by the mismatch between what the animal has previously learned to be the rate at which the US occurs during the CS and what it now observes to be the US rate, which, during extinction, converges on zero.

The information-based models are explicit in assuming that animals continuously acquire information about the rate of reinforcement (the rate at which the US occurs during the CS). During extinction, animals compute the total amount of time that the CS has been present without the US, and they stop responding when this time exceeds by some threshold the remembered waiting time to reinforcement that was learned during initial conditioning

(Gallistel & Gibbon, 2000). This means that extinction of responding will be sensitive to both the number of times that the CS is presented without the US and the length of each time that the CS is presented without the US. Indeed responding to two CSs should extinguish equally quickly as long as the CSs are matched for the cumulative amount of time that they are presented without reinforcement.¹ For example, if one CS is presented 5 times more often than another, but is five times shorter on each presentation, responding to both CSs should extinguish at the same point.

As a class, associative models are less committed in specifying the relative importance of time versus number of presentations to extinction. In presenting their model, Rescorla and Wagner (1972) specified the learning rules in a strictly trial-based fashion. In this formulation, extinction of responding would depend entirely on how often the CS has been presented without the US, and the length of those presentations would have no discernible influence. However, more recent “real-time” associative models, many of which retain the Rescorla-Wagner learning rule in describing extinction, allow associative strength to extinguish continuously during presentations of the CS (e.g., Machado, 1997; McLaren & Mackintosh, 2000; Vogel, Brandon, & Wagner, 2003; Wagner, 1981). According to these models, longer presentations of the CS without the US will facilitate extinction. Indeed, a very simple real-time version of the Rescorla-Wagner model, in which the CS is represented by a single element that has constant associability (α) across the trial, predicts that duration of CS exposure determines

¹ The information-based models typically emphasise an exclusive role for time in computing reinforcement rate as the determining factor underlying the acquisition and extinction of conditioned responding. However, the information theory framework can also be used to describe how conditioning and extinction are affected by the probability of reinforcement per trial, which mean that trials could possibly contribute to extinction beyond what they add to the cumulative time of the CS (Gallistel, 2012).

extinction, and that the number of trials only affects extinction because each trial adds to accumulated time. That is, like rate-based models, it predicts that extinction depends entirely on the cumulative duration of CS exposure. Therefore, responding will extinguish by the same amount whether the CS has been presented for a few long trials or if those long trials have been broken up into many short trials.² The importance of cumulative exposure can also be deduced from Rescorla's (1997) proposal that extinction involves the acquisition of an inhibitory S-R association, such that the loss of responding is directly proportional to the amount of responding the animal has engaged in during extinction.

A number of studies have directly investigated the importance of trials and exposure time to extinction. The first of these, by Shipley (1974), explicitly examined the role of cumulative duration of CS exposure in extinction of fear conditioning. Rats acquired fear of a tone CS as a result of 20 presentations of the CS that co-terminated with shock. The CS length was fixed at either 25 s or 100 s. The rats then received extinction trials in which the CS was presented repeatedly without shock, with each presentation being either 25 or 100-s long. The number of extinction trials varied between groups so that the cumulative duration of CS exposure was 200, 400, or 800 s. Shipley found that differences between groups in their fear responses on subsequent test were accounted for by the differences in cumulative CS exposure, and once groups were matched for cumulative exposure there was no apparent effect of trial length or number. This result provides strong support for rate-based descriptions

² This analysis is only concerned with how extinction learning proceeds during presentations of the CS, and does not consider the impact of the inter-trial interval on extinction. Some associative models, such as Wagner's (1981), predict that the length of the inter-trial interval will affect extinction. By contrast, a rate-based model like RET (Gallistel & Gibbon, 2000) assumes that the inter-trial interval does not affect extinction, even though it explicitly affects the acquisition of conditioned responding.

of extinction, as well as a simple real-time version of a prediction error model. It disconfirms any independent role of trials in extinction or any proposal that the rate of extinction learning varies at different times within the trial (e.g., Wagner, 1981). However, as we review below, this evidence has not been replicated in the three more recent studies to have addressed this topic (Drew, Yang, Ohyama, & Balsam, 2004; Golkar, Bellander, & Öhman, 2013; Haselgrove & Pearce, 2003).

Haselgrove and Pearce (2003) investigated how extinction of appetitive conditioning in rats is affected by the total amount of non-reinforced exposure to a CS. They used a conditioned magazine approach paradigm in which a CS signaled the delivery of food (the US). Conditioning was followed by extinction sessions in which the CS was presented for either short or long durations without food. In their first three experiments, when the CS was short (10 s) during training, the rate of response loss was positively affected by the length of each presentation of the CS during extinction: 30-s or 270-s presentations produced faster extinction than 10-s presentations. Importantly, the positive effect of CS length was observed even when controlling for the cumulative amount of CS exposure, which directly contradicts the findings reported by Shipley (1974). In their last two experiments, when rats were trained with a long (60-s) CS, Haselgrove and Pearce observed that response loss was inversely related to CS duration during extinction. That is, response loss was more rapid when each presentation of the CS was 10-s than when it was 60-s. In these two experiments, the two groups were matched for cumulative duration of CS exposure per session (one group was given six-times as many presentations per session to compensate for the six-fold difference in the length of each presentation). Again, these results demonstrate that the rate of extinction is not determined by

cumulative duration of non-reinforced exposure. Haselgrove and Pearce concluded that extinction is facilitated whenever the length of the trial in extinction differs from the length of the trial in conditioning, and interpreted this as showing that a change in trial length between conditioning and extinction reduces generalization of excitation from conditioning to extinction. However, what these experiments did not reveal is whether, after changing the CS duration from conditioning to extinction, responding remains extinguished or recovers when the CS is again presented at the length used during conditioning (Drew et al., 2004).

One reason that response loss would increase when the CS duration changes from conditioning to extinction is if responding is sensitive to the expected timing of the US. Timing of conditioned responses has been demonstrated across many paradigms, including fear conditioning and magazine approach (Davis, Schlesinger, & Sorenson, 1989; Drew, Zupan, Cooke, Couvillon, & Balsam, 2005; Harris, 2015; Kehoe & Joscelyne, 2005; Roberts, Cheng, & Cohen, 1989; Smith, 1968; Williams, Lawson, Cook, Mather, & Johns, 2008). In the first three experiments by Haselgrove and Pearce (2003), the faster response loss from long extinction trials compared to short ones could be explained as a simple consequence of response timing. That is, if during conditioning the rats had learned that food was delivered within 10 s of CS onset, they should stop responding during very long presentations (30 s or 270 s) once the trial exceeded the remembered time of food delivery. These considerations are relevant to the first three experiments described by Haselgrove and Pearce, but not their final two experiments. In those two experiments, the rats were conditioned with 60-s CS presentations that were reinforced with food multiple times (every 10 s), so that the rats would respond at a uniformly high rate across the length of the CS, rather than timing their responses to a single learned CS-

US interval. Therefore, the fact that many short CS exposures were more effective at extinguishing responding than a few long exposures suggests that number of trials is more important than cumulative CS exposure in controlling extinction.

In the third study to examine of the effect of CS duration on extinction, Drew et al. (2004) investigated the extinction of autoshaped key-peck responding in pigeons after conditioning with an 8-s CS-US interval. They compared the loss of responding produced by extinction trials that were shorter than (4 s), equal to (8 s), or longer than (16 s or 32 s) the conditioning interval. Response loss during extinction was not determined by cumulative CS exposure, consistent with the findings reported by Haselgrove and Pearce (2003) but not Shipley (1974). Response loss was also unrelated to CS length, except when the CS duration was much longer (32 s) than that used during conditioning and responding was measured across the entire presentation which includes a long period beyond the learned time of US delivery. Response loss was, however, affected by the number of trials. A very similar pattern has been reported in a recent study that investigated extinction of fear-potentiated startle responses to a CS that signaled shock in human subjects (Golkar et al., 2013). Fear responses were extinguished across repeated non-reinforced presentations of the CS that were either shorter (3 s), longer (24 s), or the same length (6 s) as during training. When groups were matched for cumulative duration of CS exposure during extinction, the group given many short trials showed the greatest extinction, and the group with few long trials showed the least extinction. When the data were analysed in a way that matched groups for the number of trials given under extinction, there were no longer differences in the rate of extinction between any of the groups.

Drew et al. (2004) were specifically interested in the relevance of US timing to extinction, and therefore extended each of their experiments to measure the recovery of responding on a final test session when all CS presentations returned to the conditioning duration (8 s). Under these circumstances, responding was recovered if the CS had been extinguished with short (4-s) presentations compared to groups given longer presentations during extinction, consistent with the argument that extinction is incomplete when each exposure is shorter than the remembered time of US delivery. However, this argument was contradicted by the results from one group that was given 32-s long extinction exposures yet showed significant recovery of responding when the CS duration was returned to 8 s on a final test session. Drew et al. concluded that extinction is facilitated by changes in the duration of the CS from conditioning to extinction because this reduces generalization of excitation from conditioning to extinction, but a return to the conditioning duration results in a recovery of responding.

In summary, whereas Shipley (1974) found that extinction was determined entirely by the cumulative duration of non-reinforced exposure to the CS, Haselgrove and Pearce (2003), Drew et al. (2004), and Golkar et al. (2013) found that extinction was related to the number of non-reinforced trials but did not depend on cumulative CS exposure. In the experiments reported by Haselgrove and Pearce and Drew et al., any effect of CS duration on extinction was clearly related to the CS-US interval. Both groups found that response loss during extinction was positively related to CS duration when the CS extended well beyond the CS-US interval used during conditioning, an effect that could reasonably be attributed to the cessation of responding when time in the CS exceeds the remembered time of US delivery. Haselgrove and

Pearce (2003) also found that response loss was facilitated by making the CS much shorter during extinction than during conditioning, but this effect was confounded in their design by an increase in the number of extinction trials for the short CS. When Drew et al. (2004) tested the effect of reducing the CS duration in extinction, without changing the number of trials, they found CS duration did not affect the rate of response loss but did reduce the effectiveness of extinction when CS length was returned to the conditioning duration. Therefore, it remains unclear whether the length of the CS affects extinction when the length does not exceed the maximum CS-US interval used in conditioning. This is an important issue because any model of learning that assumes extinction is continuous during a trial would predict that longer CS presentations produce more extinction than shorter ones.

Here we describe four experiments which, like the ones reviewed above, examined the importance of trial number, CS length, and cumulative CS exposure on extinction. However, unlike most of the previous experiments, they avoid the complicating factor of response timing that arises when animals are conditioned with a single fixed CS-US interval. In the experiments we present here, rats were conditioned using randomly varying CS-US intervals so that they could not learn to anticipate the US at a specific time after CS onset, and could not time their responses accordingly. We then extinguished responding, and compared response loss between CSs that differed in the length and/or number of extinction trials. When examining the effect of CS length on extinction, we did not test lengths that exceeded the maximum CS-US interval used during conditioning, so that any effect of CS length on extinction was not confounded by response timing or temporal changes in US expectancy. By making expectancy

of the US uniform across the length of the CS, our experiments should provide the optimal conditions to observe an effect of the duration of CS exposure on extinction.

Experiment 1

Experiment 1 compared extinction of responding to two CSs, A and B, that had undergone equivalent conditioning using a variable CS-US interval between 2 and 38 s (mean = 20 s). There were five presentations of A per extinction session, and the duration of these matched the range of intervals used during conditioning (i.e., a mean length of 20 s). There were 20 presentations of B per session, and the duration of these was short (mean = 5 s). Thus both CSs were presented for a total duration of 100 s per session. If extinction is determined by total duration, as reported by Shipley (1974), then responding to A and B should extinguish at the same rate across sessions. However, based on the findings reported by Haselgrove and Pearce (2003) and Drew et al. (2004), we should expect to see more rapid extinction of responding to B than A due to the difference in their number of trials.

Our experiments differed from the previous ones in a number of ways. As already mentioned, we used variable CS-US intervals during conditioning to prevent timing of conditioned responses, and when manipulating CS length during extinction, we did not give long CS presentations that extended beyond the maximum CS-US interval used in conditioning. Further, our experiment used an entirely within-subjects design, whereas all of the previous work used between-subjects designs. There are two distinct advantages to within-subjects designs. The first is that they avoid confounds related to potential differences in baseline

responding between groups. The second is that they control for the large variance that can exist between rats in their levels of responding, and therefore can be more sensitive in detecting differences between conditions. Finally, we included two control CSs, C and D, that were conditioned at the same time as A and B, and continued to be reinforced with food throughout the extinction sessions for A and B. This was done so that the loss of responding (magazine entry) during A and B would reflect extinction of those CSs specifically, rather than extinction of the magazine that might otherwise mask differences in extinction of the two CSs.

Subjects

Sixteen experimentally-naïve female albino Sprague Dawley rats (8 to 10 weeks of age at the start of the experiment) were obtained from 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 the School of Psychology animal colony. The rats had unrestricted access to water in the home boxes. 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).

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 high-pitch tone (2.9 kHz, 17 dB above background) was produced from a piezo buzzer positioned on the floor of the sound-attenuating shell behind each conditioning chamber. A flashing light (2 Hz; 3.0cd/m²) was emitted by an array of white LEDs, located on the floor of the sound-attenuating shell in front of the operant chamber. A steady light (30cd/m²) was produced by an incandescent bulb mounted high on the back wall of the sound-attenuating shell. Allocation of the four stimuli to the four CSs was counterbalanced between rats with the following constraints: A and B were from different modalities, as were C and D. These constraints increased the discriminability between A and B.

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 60 trials, 15 trials of each of CS. CS durations varied randomly from trial to trial, sampled from a uniform distribution of durations between 2 and 38 s (mean = 20 s). A single food pellet was delivered at the termination of the CS on every trial. Trials of each type were randomly intermixed. The inter-trial interval varied randomly with a mean of 110 s, and the session lasted 2 h and 10 min.

Sessions 21 to 35 each contained 65 trials, consisting of 5 trials of A and 20 trials of each of B, C, and D. Trials with A had a mean length of 20 s (2 to 38 s) and were not reinforced with food; trials with B had a mean length of 5 s (2 to 8 s) and were not reinforced with food; trials with C and D had a mean length of 20 s and were reinforced with food. Photo-beam interruptions by head entry into the magazine were recorded during each CS and each 20-s pre-CS period for all 35 sessions.

Results and Discussion

Mean response rates across all 35 sessions of Experiment 1 are presented in the top panel of Figure 1. Responding to the four CSs increased across Sessions 1 to 20. There was little difference in response rates between the CSs, consistent with the fact that they were all given

the same schedule of reinforcement. We conducted an Analysis of Variance (ANOVA), with Greenhouse-Geisser correction to the degrees of freedom when the data failed the test for sphericity, on the data from Sessions 1 to 20. This confirmed that there was a significant main effect of trial period (during the four CSs and the pre-CS periods), $F(1, 15) = 48.02, p < .001, \eta^2_p = 0.76$ [95% confidence intervals (CIs) on $\eta^2_p = 0.69$ and 0.87 ; (Hentschke & Stüttgen, 2011)]. This main effect was driven by a significant difference in response rate, averaged across the 20 sessions, between the four CSs and the pre-CS period, $F(1, 15) = 56.28, p < .001, \eta^2_p = 0.79$ [0.71 and 0.91]. There was also a significant main effect of Session, $F(2.5, 38) = 5.28, p = .006, \eta^2_p = 0.26$ [0.17 and 0.47], which interacted with the main effect of trial period, $F(4.2, 63) = 5.15, p = .001, \eta^2_p = 0.26$ [0.20 and 0.41].

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

Across Sessions 21 to 35, responding to A and B declined while responding to C and D remained elevated, consistent with the fact that A and B were no longer reinforced but C and D were still reinforced. An ANOVA on the data from Sessions 21 to 35 confirmed that there was a significant difference overall between the non-reinforced CSs (A and B) and the reinforced CSs (C and D), $F(1, 15) = 25.69, p < .001, \eta^2_p = 0.63$ [0.49 and 0.79]. There was also a significant main effect of Session, $F(2.9, 44) = 4.11, p = .012, \eta^2_p = 0.22$ [0.16 and 0.47], that interacted with the main effect of reinforcement, $F(3.3, 49) = 9.46, p < .001, \eta^2_p = 0.39$ [0.30 and 0.57]. The comparison of primary interest is between responding to A and responding to B across these extinction sessions. It is clear from Figure 1 that responding to B declined more rapidly than did responding to A. This was also confirmed by ANOVA, which showed there to be a significant

difference overall between A and B, $F(1, 15) = 21.77, p < .001, \eta^2_p = 0.59$ [0.47 and 0.75]. There was a significant overall effect of Sessions, $F(3.7, 56) = 10.23, p < .001, \eta^2_p = 0.41$ [0.34 and 0.58], but this did not interact significantly with the difference between A and B after Greenhouse-Geisser correction, $F(4.4, 66) = 2.38, p = .055$.

The analysis described above compares response rates measured across relatively long presentations of A (on average 20-s long) with response rates measured across much shorter presentations of B (on average 5-s long). While response rates tend to be steady across the length of the trial when the CS-US interval is variable (Harris & Carpenter, 2011; Harris, Gharaei, & Pincham, 2011), as we have implemented here, response rates do change substantially in the first few seconds of each trial. The time taken for response rates to rise at the start of each trial will have a greater impact on the overall response rate for very short trials than for longer trials, and this may have contributed to the difference in responding between A and B, described above. To take account of this possibility, we have recalculated the response rates to the longer CS, A, by measuring responses made during just the first quarter of each trial. This means we are comparing responses to A and B across time periods with the same length (a mean duration of 5 s). The response rates during the first quarter of each A trial are shown by the dashed black line in the top panel of Figure 1. It is clear that there is still a difference between A and B. This difference is confirmed by an ANOVA which showed that the difference overall between A and B was significant, $F(1, 15) = 38.84, p < .001, \eta^2_p = 0.72$ [0.62 and 0.85], as was the interaction between this difference and the change in responding across sessions, $F(4.1, 62) = 2.63, p = .042, \eta^2_p = 0.15$ [0.13 and 0.29].

The more rapid extinction of responding to B than to A shows that extinction is affected by the number of CS presentations even when controlling for differences in the overall amount of exposure to the CS. The fact that A's extinction trials were five times longer than B's did not compensate for the fact that there were five times as many B trials as A trials. This shows that number of trials has a larger effect on extinction than duration of trials, but does not in itself show that the duration of the trial is without effect on the rate of extinction. Another way to assess the relative importance of number and length of trials is to compare extinction of A and B per trial rather than per session. If the duration of the trial does influence the rate of extinction, we would expect to see more rapid extinction of A than B when response rates are plotted per trial since this now equates the two CSs in terms of the influence of trial number on extinction. Such a comparison is shown in the lower panel in Figure 1. This shows the response rates to A and B, across sessions 21 to 35, averaged in blocks of five consecutive trials. (Averaging across five consecutive trials still equates A and B for the effect of trial number, but reduces a lot of the variability that exists in single-trial response rates.) It is striking how similar the response rates are to A and B across the first 15 data points, corresponding to all 75 trials of A and the first 75 trials of B. Responding to both CSs changed little for the first 40 trials, and then decreased at an almost identical rate across the next 35 trials. This is remarkable given that these 75 trials were spaced across 15 sessions for A but occurred within the first 4 sessions for B. An ANOVA comparing A and B across these first 15 5-trial blocks showed there was a significant main effect of session, $F(3.7, 56) = 8.54, p < .001, \eta^2_p = 0.36$ [0.32 and 0.50], but no overall difference between A and B, $F(1, 15) = 0.10, p = .76$, and no interaction between session and A versus B, $F(2.8, 42) = 0.50, p = .670$.

We followed the analysis just described by calculating a Bayes Factor for the comparison between the response rates to A and B averaged over the first 75 trials. This compared the likelihood of the null hypothesis, that A and B are equal, with the likelihood that responding to A is less than responding to B (which should be true if the extra duration of A's presentations accelerates its extinction). To derive a marginal likelihood of this alternative hypothesis, we specified that the difference between A and B should be at least 0.02 responses /s (i.e., 5% of the overall mean response rate) and not more than 0.2 responses /s (50% of the overall mean response rate). This gives a Bayes Factor of 3.75, which means that, given the data, it is 3.75 times more likely that A and B are equal than that they differ.

Experiment 2

Experiment 1 has shown that the number of non-reinforced trials per session has a large effect on the rate of extinction, even when controlling for the duration of exposure to the CS. However, when controlling for the effect of number of trials, there was no evidence that trial duration affected the rate of extinction. Experiment 2 investigated this second issue further. It gave rats equivalent training with two CSs, A and B, using variable CS-US intervals that had a mean of 40 s (range 2 to 78 s). Responding to both CSs was then extinguished. During extinction, the two CSs were matched for number of trials per session, but differed in length: each presentation of A lasted 15 s, and each presentation of B lasted 75 s. The aim was to test whether this fivefold difference in duration produced any difference in the rate of extinction. As a positive control, to establish that the rats are sensitive to this difference in CS duration, the extinction phase included training with two new CSs, C and D, that were given an equal number

of reinforced trials in each session but differed in the mean duration of those trials (15 s versus 75 s). Based on our previous work (Harris & Carpenter, 2011), we expected that the rats would acquire much higher levels of responding to the shorter of these CSs than the longer. The question of primary interest was whether this difference in acquisition would be mirrored by a difference in extinction.

Subjects

Sixteen experimentally-naïve female Hooded Wistar rats (8 to 10 weeks of age at the start of the experiment) were obtained from the Laboratory Animal Services breeding unit at The University of Adelaide, South Australia. During the experiment, they were housed in groups of 8 in large white plastic tubs, measuring 26 x 59 x 37cm (height x length x depth), located in the School of Psychology animal colony maintained by Laboratory Animal Services at the University of Sydney. They 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, as described for Experiment 1.

Apparatus

Rats were trained and tested in the 16 conditioning chambers described previously. The four stimuli used in Experiment 1 (white noise, tone, flashing light, and steady light) were used as CSs A to D, and were allocated in counterbalanced fashion with the constraint that A and B were from different modalities, as were C and D. This experiment also included a clicker stimulus (4 clicks per second, each click 24 dB above background) emitted from a module (Med Associates, product ENV 135M) located on the wall of the conditioning chamber above and to

the right of the magazine. The clicker, labelled as stimulus X, and was not counterbalanced. Its purpose is explained below.

Procedure

Rats first received magazine training as described for Experiment 1. They then commenced daily conditioning sessions 5 days per week for a total of 35 sessions. Each of the first 18 sessions contained 36 trials, made up of 12 trials of each of A, B and X. Presentations of A and B had a mean duration of 40 s (uniformly distributed in the range from 2 to 78 s) and were followed immediately by delivery of a single food pellet. Presentations of X were also 40 s (2 to 78 s) but were never reinforced with food. They were included so that the rats were not always reinforced (regardless of CS), in the hope that this would increase the likelihood that the rats would discriminate between A and B. Trials of each type were randomly intermixed, and the inter-trial interval varied randomly with a mean of 110 s. Each session lasted approximately 1 h and 30 min.

On Sessions 19 and 20, A and B continued on the same reinforcement schedule as for the previous sessions. Stimulus X was no longer presented, and instead each session included 12 presentations of each of two new CSs, C and D. These two CSs had a mean duration of 40 s (range 2 to 78 s). On these two sessions, C and D were not followed by food. They were presented in this way to reduce generalization of responding from A and B to C and D.

On Session 21, the rats commenced extinction training with A and B, while concurrently receiving conditioning with C and D. For the next 14 sessions, all four CSs were presented 12 times each. A was presented for a fixed duration of 15 s, and never followed by food. B was

presented for a fixed duration of 75 s, and never followed by food. C was presented for a variable duration with a mean of 15 s (2 to 28 s) and always followed by food. D was presented for a variable duration with a mean of 75 s (2 to 148 s) and always followed by food. This schedule meant that CS duration could not be used to determine whether a trial would or would not be reinforced.

The final session, Session 35, was designed to test whether the rats could discriminate between A and B, by re-conditioning A before testing response rates to A and B. The session began with 20 trials in which A was presented with a variable duration of 15 s and always reinforced with food. The conditioning trials of A were intermixed among 10 reinforced trials with C and D, as on Days 21 to 34. These 40 trials were immediately followed by six trials of A, B, C and D, intermixed. A and B were both presented with a fixed duration of 15 s and not reinforced; C and D were reinforced according to their previous schedules. If, across the course of the experiment, the rats had come to treat A and B as equivalent stimuli, we would expect to see similar levels of response recovery to A and B on this final test phase. Throughout the experiment, photo-beam interruptions by head entry into the magazine were recorded during each CS and each 20-s pre-CS period.

Results and Discussion

Mean response rates across all 35 sessions of Experiment 2 are shown in Figure 2. The rats acquired responding to A and B across the first 20 sessions, and were responding during these CSs much more than in the pre-CS period or during the non-reinforced stimulus X. An ANOVA confirmed that responding to A and B was significantly higher than during the pre-CS period,

$F(1, 15) = 61.15, p < .001, \eta^2_p = 0.80$ [CI = 0.65 and 0.94], but there was no difference between A and B, $F(1, 15) = 0.08, p = .778$.

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

From Sessions 21 to 34, responding to A and B extinguished, while responding to C and D increased. A $2 \times 2 \times 14$ ANOVA showed that responding to the two CSs undergoing extinction (A and B) was significantly different from responding to the two CSs undergoing conditioning (C and D), $F(1, 15) = 63.97, p < .001, \eta^2_p = 0.81$ [0.64 and 0.93]. This difference interacted significantly with the effect of sessions, $F(2.5, 36) = 40.66, p < .001, \eta^2_p = 0.73$ [0.64 and 0.83]. There was also a significant difference between the two long CSs (B and D) and the two short CSs (A and C), $F(1, 15) = 109.96, p < .001, \eta^2_p = 0.88$ [0.75 and 0.96], which interacted with the effect of sessions, $F(6.6, 100) = 5.93, p < .001, \eta^2_p = 0.28$ [0.17 and 0.48]. There was a significant interaction between the effects of reinforcement (extinction versus conditioning) and CS length, $F(1, 15) = 60.60, p < .001, \eta^2_p = 0.80$ [0.60 and 0.95], which formed a three-way interaction with sessions, $F(4.5, 67) = 11.68, p < .001, \eta^2_p = 0.44$ [0.35 and 0.58].

We conducted two separate 2×14 ANOVAs on the CSs undergoing extinction (A and B) and the CSs undergoing conditioning (C and D). When analyzing data from the entire length of trials with CS B, there was a significant difference overall between responding to A and responding to B, $F(1, 15) = 18.18, p = .001, \eta^2_p = 0.55$ [0.30 and 0.78]. There was also a significant effect of sessions, $F(4.3, 65) = 26.68, p < .001, \eta^2_p = 0.64$ [0.53 and 0.77], which interacted with the difference between A and B, $F(5.5, 82) = 2.44.96, p = .036, \eta^2_p = 0.14$ [0.09 and 0.34]. Because this analysis compares responding to A versus B across very different time

windows, we reanalyzed the response rates to B using only the first 15 s of each trial in order to match these with the length of the A trials. As is clear in Figure 2, the response rates during the first 15 s of each B trial were very similar to the response rates to A. An ANOVA on these response rates showed there was no overall difference between A and B, $F(1, 15) = 0.21$, $p = .655$, nor was there an interaction with sessions, $F(3.9, 58) = 1.03$, $p = .426$, but there was a main effect of sessions, $F(4.2, 64) = 24.31$, $p < .001$, $\eta^2_p = 0.62$ [0.50 and 0.75].

An ANOVA comparing response rates to C and D (all 75 s of each trial) on Sessions 21 to 34 confirmed that there was a significant overall difference between the two CSs, $F(1, 15) = 91.90$, $p < .001$, $\eta^2_p = 0.86$ [0.71 and 0.96]. There was also a significant main effects of sessions, $F(3.5, 53) = 8.80$, $p < .001$, $\eta^2_p = 0.37$ [0.30 and 0.59], and a significant interaction between CS and sessions, $F(5.3, 79) = 19.46$, $p < .001$, $\eta^2_p = 0.57$ [0.48 and 0.69]. The response rates to D changed little when only the first 15 s of each trial were analysed, and the outputs of the ANOVA using these data were very similar to those just reported.

We conducted a Bayesian analysis to follow up the comparison between response rates during A and during the first 15 s of B, averaged over Sessions 21 to 34. This analysis compared the likelihood that response rates to A and B were equal versus the likelihood that response rates to B would be less than to A (which should be observed if the longer presentations of B produced more extinction). The Bayes Factor always favoured the null ($A=B$) over the alternative for any assumed size of effect for $A>B$. Even for a difference as small as 0.02 response /s, the Bayes Factor gave an odds ratio of 24 favouring the null, and these odds increased for any increase in the assumed difference between A and B.

Experiment 3

The two previous experiments have shown that extinction is positively affected by the number of non-reinforced trials but does not appear to be affected by differences in the length of those trials (whereas acquisition was affected by CS length). Experiment 3 was designed to provide a within-subject comparison of the effects of trial number versus total CS duration on extinction. Two groups were trained with four CSs before responding to the CSs was extinguished. Two of the CSs (A and B) were matched for their total duration per session but they differed in the number of trials per session. The other two CSs (C and D) were matched on the number of extinction trials per session but differed in their total duration. Based on the results from Experiments 1 and 2, we expected a larger difference in the rate of extinction between A and B than between C and D.

The two pairs of CSs were conditioned with different rates of reinforcement (one pellet per 15 s versus one pellet per 30 s) to reduce generalisation between the CSs that might otherwise obscure differences in rates of extinction. This difference in conditioning between the CS pairs was counterbalanced across the two groups of rats. Thus, for Group 1, CSs A and B were conditioned with the higher rate of reinforcement, while C and D were reinforced at the lower rate. For Group 2, A and B were conditioned at the lower reinforcement rate while C and D were conditioned at the higher rate.

There is a problem when comparing rates of extinction between CSs that differ in trial length. If responding is measured across the full length of each extinction trial, then response

rates are collected across a short time window for one CS but a long time window for the other CS. Thus the effect of trial length on learning during extinction is confounded with its effect on responding at test. This issue was addressed in Experiments 1 and 2 by comparing responding to the shorter CS with responding during just the initial portion of each trial of the longer CS. This strategy matched the two CSs in terms of how response rates were collected. However, it only tells us whether the CSs differ during that first part of the full CS. For example, in Experiment 2, CSs A and B were reinforced at variable times between 2 and 78 s during conditioning, before being extinguished with either 15-s or 75-s trials. Responding during the first 15 s of the trial extinguished at very similar rates for the two CSs, showing that the much longer presentations of B did not increase evidence for extinction during those first 15-s. However, we cannot know from this whether responding to A and B would continue to look the same, or would in fact differ, if we had measured responding beyond those first 15 s of the trial. For example, according to some componential models which treat the CS as an array of microelements distributed in time (Machado, 1997; Vogel et al., 2003), responding to A should be higher than B during long presentations because the short trials with A would not extinguish late CS elements that are only activated on long trials. To address this problem, each extinction session of Experiment 3 included two full length probe trials of the short CS. For example, in Group 1, most extinction trials with A were 10-s long, compared with 30 s for B, but each session included two 30-s trials with A. Only these full-length trials were used when comparing response rates between A and B. Moreover, to further match the CSs, the recording of responses to B was downsampled, using only the two trials that fell closest during the session to the two full length probe trials with A.

Subjects and Apparatus

Thirty two experimentally-naïve female Hooded Wistar rats (8 to 10 weeks of age at the start of the experiment) were obtained from the Laboratory Animal Services breeding unit at The University of Adelaide, South Australia. They were housed in the same manner and under the same condition as described for Experiment 2. The rats were trained and tested in the 16 conditioning chambers described previously. The white noise, tone, flashing light, and steady light described in Experiment 1 were used as CSs A to D, in counterbalanced fashion. As for Experiment 2, the clicker served as stimulus X, and was not counterbalanced.

Procedure

Rats first received magazine training as described previously, and then commenced daily conditioning sessions 5 days per week for a total of 28 sessions. Each of the first 20 sessions contained 60 trials, 12 trials of each of the five stimuli (A, B, C, D, and X). Two CSs (A and B in Group 1, C and D in Group 2) had a mean duration of 15 s (from a uniform distribution between 2 and 28 s). The other two CSs (C and D in Group 1, A and B in Group 2) had a mean duration of 30 s (range 2 to 58 s). Each presentation of these four CSs was followed immediately by delivery of a single food pellet. Presentations of X were 15 s (2 to 28 s) but were not reinforced with food. Trials of each type were randomly intermixed, and the inter-trial interval varied randomly with a mean of 120 s. The session lasted approximately 2 h and 20 min.

On Sessions 21 to 28, the rats were split into two groups (16 rats each) before commencing extinction training with CSs A to D. For both groups, A and B differed in the number of trials per session but were matched on total duration. For Group 1, there were 12

trials of A, all 30-s long, and 32 trials with B, of which 30 were 10-s long and 2 were 30-s long. For Group 2, there were 12 trials with A, all 60-s long, and 32 trials with B, of which 30 were 20-s long and 2 were 60-s long. Thus, in both groups, there were $2\frac{2}{3}$ times as many B trials as A trials, but both CSs were presented for the same total time per session (360 s for Group 1, 720 s for Group 2). For both groups, CSs C and D had the same number of trials per session, but D trials were longer than C trials, such that the two CSs differed in total duration by a factor of $2\frac{2}{3}$. For Group 1, there were 12 trials with C, each 60-s long (total durations = 720 s), and 12 trials with D, of which 10 were 15-s long and 2 were 60-s long (total duration = 270 s). For Group 2, there were 12 trials with C, each 30-s long (total duration = 360 s), and 12 trials with D, of which 10 were 7.5-s long and 2 were 30-s long (total duration = 135 s). In both groups, all presentations of CSs A to D ended without reinforcement. Throughout these extinction sessions, stimulus X was now presented with reinforcement at the end of each presentation (mean duration 15 s). X was reinforced in order to maintain food delivery in the magazine throughout these sessions. Photo-beam interruptions by head entry into the magazine were recorded during each CS and each 20-s pre-CS period for the entire experiment.

Results and Discussion

Mean response rates during each CS across the 28 sessions of Experiment 3 are shown for both groups in Figure 3. Across the first 20 sessions, the rats acquired responding to the four reinforced CSs (A to D) while showing very little responding during X which was not reinforced. At this time, in both groups, the two CSs that were reinforced after an average duration of 15 s (A and B for Group 1, C and D for Group 2) elicited more responding than did the two CSs that

were reinforced after an average of 30 s (C and D for Group 1, A and B for Group 2). An ANOVA on the data from Sessions 1 to 20 showed there was a significant difference between responding during the pre-CS period and responding during the four reinforced CSs, $F(1, 30) = 197.70$, $p < .001$, $\eta^2_p = 0.87$ [CI = 0.82 and 0.93]. This difference did not interact across the two groups, $F(1, 30) = 0.22$, $p = .640$. A $2 \times (2) \times (2) \times (20)$ ANOVA on the response rates during CSs A, B, C and D, showed there to be a significant effect of reinforcement rate (mean wait of 15 s versus 30 s), $F(1, 30) = 68.82$, $p < .001$, $\eta^2_p = 0.69$ [0.60 and 0.82], and a significant effect of Session, $F(3.1, 93) = 26.79$, $p < .001$, $\eta^2_p = 0.47$ [0.39 and 0.58], and a significant interaction between these factors, $F(4.4, 133) = 4.88$, $p = .001$, $\eta^2_p = 0.14$ [0.09 and 0.24]. The effect of reinforcement rate did not interact with Group, $F(1, 30) = 0.12$, $p = .727$, nor was there an interaction between Group and Session, $F(3.1, 93) = 0.50$, $p = .964$. The three-way interaction between Group, reinforcement rate, and Session, was significant, $F(4.4, 133) = 2.56$, $p = .036$, $\eta^2_p = 0.08$ [0.05 and 0.19], but was judged to be of no consequence.

The important results are from the extinction Session, (Session 21 to 28) shown on the right side of Figure 3. A $2 \times (2) \times (2) \times (8)$ ANOVA compared extinction during the four critical CSs (A to D). In the interests of clarity, we will report here only the theoretically meaningful parts of this analysis. There was a significant overall effect of Sessions, $F(2.1, 64) = 61.20$, $p < .001$, $\eta^2_p = 0.67$ [0.60 and 0.74]. The difference in response rate during conditioning between the two CSs reinforced at a high rate (15 s) and those reinforced at a lower rate (30 s) persisted during the extinction sessions, $F(1, 30) = 43.39$, $p < .001$, $\eta^2_p = 0.59$ [0.50 and 0.73], and this interacted with Sessions, $F(3.9, 118) = 13.41$, $p < .001$, $\eta^2_p = 0.31$ [0.20 and 0.47]. The difference between the CSs reinforced at the higher versus lower rate during conditioning also interacted with

Group, $F(1, 30) = 6.94$, $p = .013$, $\eta^2_p = 0.19$ [0.03 and 0.40]. The most important result is the interaction comparing the difference between A and B with the difference between C and D, across both groups. This was significant, $F(1, 30) = 14.55$, $p = .001$, $\eta^2_p = 0.33$ [0.13 and 0.57], showing that the difference between responding to A and responding to B was larger than the difference between responding to C and responding to D. This interaction also interacted significantly with Sessions, $F(2.9, 87) = 4.38$, $p = .007$, $\eta^2_p = 0.13$ [0.04 and 0.33], but did not interact with Group, $F(1, 30) = 1.70$, $p = .202$. Therefore, extinction was affected significantly more by a difference in the number of trials than a difference in the duration of exposure.

To unpack these results, the four factor ANOVA was followed up with separate (2)x(2)x(8) ANOVAs on each group. These confirmed that there was a significant interaction between the first two factors in each group: for Group 1, $F(1, 15) = 10.02$, $p = .006$, $\eta^2_p = 0.40$ [0.10 and 0.72]; for Group 2, $F(1, 30) = 4.55$, $p = .050$, $\eta^2_p = 0.23$ [0.03 and 0.50]. In other words, the difference in responding between A and B was larger than the difference between C and D. This interacted itself with Sessions in Group 1, $F(2.1, 31) = 4.04$, $p = .026$, $\eta^2_p = 0.21$ [0.07 and 0.51], but not in Group 2, $F(3.6, 54) = 1.48$, $p = .224$. Finally, the interaction in each group was explored using two separate (2)x(8) ANOVAs, one comparing A against B, the other comparing C against D. The difference between A and B was significant in both groups: for Group 1, $F(1, 15) = 9.54$, $p = .007$, $\eta^2_p = 0.39$ [0.08 and 0.73]; for Group 2, $F(1, 15) = 16.61$, $p = .001$, $\eta^2_p = 0.53$ [0.32 and 0.72]. The difference between A and B also interacted with Sessions: for Group 1, $F(2.2, 33) = 5.25$, $p = .009$, $\eta^2_p = 0.26$ [0.10 and 0.52]; for Group 2, $F(3.0, 45) = 4.47$, $p = .008$, $\eta^2_p = 0.23$ [0.13 and 0.45]. By contrast, there was no overall differences between C and D in either group: for Group 1, $F(1, 15) = 2.23$, $p = .150$; for Group 2, $F(1, 15) = 0.98$, $p = .338$. The

comparison between C and D did not interact with Sessions: for Group 1, $F(1, 15) = 0.95$, $p = .474$; for Group 2, $F(3.3, 49) = 1.15$, $p = .339$. Therefore, this experiment confirmed the results of Experiments 1 and 2 in showing that differences in trial number affect the rate of extinction, but differences in duration of exposure do not.

Experiment 4

The previous experiments have shown that extinction is sensitive to the number of times the CS is presented but not the duration of those presentations. Indeed, when comparing two CSs undergoing extinction, if responding was measured over a comparable interval for both CSs (i.e., either both short, as in Experiment 2, or both long, as in Experiment 3), the rate of extinction across trials was the same, even if the presentations of one CS were much longer than presentations of the other. Therefore, in these experiments, learning during extinction occurred on a trial-by-trial basis, as if the CS-US association was updated at only one point per trial rather than developing continuously while the CS was present without reinforcement. The importance of trials rather than time may have arisen due to a particular feature of the conditioning protocol used here. In all of the experiments thus far, conditioning involved the delivery of a food pellet at the termination of the CS and never during the CS. As a result, the rats may have specifically learned about the relationship between CS offset and the US, and all responses made during the CS may have occurred either via second order conditioning between the CS and its offset or via generalization from the features of CS offset to the features of the CS presentation. Therefore, extinction of responding may depend on

extinction of the association between CS offset and the US, which would occur only once per trial and would not be affected by the duration of the CS presentation.

Experiment 4 re-examined the possibility that extinction would be affected by the total duration of CS exposure, but used a conditioning protocol that prevented rats from learning an association between CS offset and the US. The rats were trained with two CSs, A and B, that had a fixed duration of 40 s but were reinforced once per trial at a variable time during the presentation. The mean interval between CS onset and food was 20 s. A third CS, X, was concurrently conditioned (on a VT 10-s schedule, with a fixed duration of 20 s), and a fourth CS, Y, was presented without reinforcement in order to prevent rats from learning that all trials were reinforced regardless of the stimulus. After 25 days of training, responding to CSs A and B was then extinguished using an equal number of trials per session, while X continued to be reinforced as before. Extinction trials with A were 10-s long, and trials with B were 40-s long. Each extinction session included one long (40 s) trial with A, and response rates to A were calculated from this trial alone for the purposes of comparison with response rates to B. If, as suggested above, our previous findings arose because the rats had specifically learned the relation between the CS offset and the US, then those results would not be replicated here. As such, we predicted that extinction would be sensitive to trial duration, and that responding to CS B would extinguish faster than responding to CS A because the total duration of exposure to B was much longer.

Subjects and Apparatus

Sixteen experimentally-naïve female Sprague Dawley rats (8 to 10 weeks of age) were obtained from the Animal Resources Centre, Perth, Western Australia. They were housed in groups of four in split-level ventilated plastic tubs, as in Experiment 1. They 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, as described for Experiment 1. The rats were trained and tested in the 16 conditioning chambers described previously. Four stimuli (white noise, steady light, tone, and clicker, described previously) were used.

Procedure

Rats first received magazine training as described for Experiment 1. They then commenced daily conditioning sessions 5 days per week for a total of 35 sessions. Each of the first 25 sessions contained 36 trials, made up of 9 trials with each of the four stimuli. Two CSs, A and B (white noise and light, counterbalanced), were presented for a fixed duration of 40 s, with a single food pellet being delivered at a variable time during the presentation. The mean time of food was 20 s (times were uniformly distributed in the range 2 to 38 s). The other two stimuli, X (clicker) and Y (tone) were presented for a fixed duration of 20 s. A single food pellet was delivered on a VT 10 s (range 2-18 s) during each presentation of X. Presentations of Y were never paired with food. The non-reinforced Y trials were included to encourage the rats to discriminate between different trial types. Trials of each type were randomly intermixed, and the inter-trial interval varied randomly with a mean of 150 s. The session lasted approximately 1 h and 50 min.

On Sessions 26 to 35, A and B were presented without food. B was presented 9 times per session for a fixed duration of 40 s. A was presented 8 times for a fixed duration of 10 s, and once for 40 s, per session. This meant that the total duration of A's presentations per session was three times longer than the total duration of B's presentations (360 s versus 120 s). During these sessions, X continued on the same reinforcement schedule as for the previous sessions, but was presented 18 times per session. Y was no longer presented. Throughout the experiment, photo-beam interruptions by head entry into the magazine were recorded during each CS and each 20-s pre-CS period.

Results and Discussion

The mean response rates across all 35 sessions of Experiment 4 are shown in Figure 4. Over the first 25 sessions, the rats learned to respond to the three reinforced CSs, A, B, and X, and responded very little to the non-reinforced CS, Y. A (3)x(25) ANOVA was used to confirm that the rats were responding reliably to A and B. This showed that response rates to A and B, averaged over all 25 sessions, were significantly different from the pre-CS period, $F(1,15) = 65.04$, $p < .001$, $\eta^2_p = 0.81$ [CI = 0.72 and 0.93]. There was a significant main effect of Sessions, $F(6.5,23) = 4.19$, $p = .005$, $\eta^2_p = 0.22$ [0.14 and 0.41], that interacted with CS, $F(5.0,75) = 4.48$, $p = .001$, $\eta^2_p = 0.23$ [CI = 0.17 and 0.44]. There was also an unexpected difference between A and B, $F(1,15) = 5.34$, $p = .036$, $\eta^2_p = 0.26$ [0.01 and 0.66], with response rates to A being slightly higher than to B. Given that A and B received identical treatment across those 25 sessions, we will not consider this difference further.

On Sessions 26 to 35, responding to C and D was extinguished. For the analysis reported here, we used response rates measured during the one 40-s long probe trial with A per session, so that they could be compared with response rates to B collected across the same 40-s time window. A (2)x(10) ANOVA compared responding to A and B across the 10 extinction sessions. This showed there was a significant effect of Session, $F(3.4,52) = 13.06$, $p < .001$, $\eta^2_p = 0.47$ [0.40 and 0.63], but there was not a significant difference overall between A and B, $F(1,15) = 0.12$, $p = .731$. Nor was there an interaction between A versus B and Sessions, $F(3.8,57) = 0.71$, $p = .583$. When the analyses were run using response rates collected from all A trials (not just the one 40-s long trial), there was still no significant difference between A and B, $F(1,15) = 2.02$, $p = .176$, nor an interaction with Sessions, $F(3.3,49) = 1.89$, $p = .140$. Therefore, just as had been observed in the previous experiments, this experiment failed to find any evidence that increasing the duration of CS exposure during extinction had any impact on the rate of response loss, even when the US was delivered during rather than at the end of the CS during conditioning.

General Discussion

The four experiments presented here show that the rate at which responding extinguishes is directly affected by the number of extinction trials but is not affected at all by the cumulative duration of CS exposure during extinction. In Experiment 1, responding extinguished more rapidly to a CS that was given many short extinction trials than a CS given few long trials, even though the two CSs were matched for the total time of their exposure per

extinction session. A particularly striking result was obtained in this experiment when response rates were plotted so as to match the two CSs for trial number. In this case, responding to the two CSs extinguished at almost identical rates, despite the fact that this analysis introduced large differences between the CSs in their cumulative exposure duration and trial density. This implies that extinction was strictly determined by trial number, and duration of exposure had no influence on extinction.

Experiment 2 confirmed the second implication of Experiment 1's results. It showed that, for two CSs given the same number of extinction trials per session, extinction developed at the same rate despite a fivefold difference in the length of their trials. This observation—that extinction was unaffected by trial length—depended on using the same time window of CS presentation when comparing response rates between the CSs. That is, in Experiment 2, there was no difference in extinction between a long CS and a short CS if responding to the long CS was measured across a short period at the start of each presentation, comparable to the length of the short CS. However, this result did not depend on truncating measurement of responding to the long CS. Experiment 3 also found no difference in extinction between a CS given short presentations and one given long presentations, and in this case measurement of responding to the short CSs was matched to the long CS by including a small number of long probe trials with the short CS. Thus, Experiment 3 showed that a large difference in total CS duration does not affect extinction, but at the same time this experiment confirmed that an equivalent difference in number of trials did affect extinction.

Experiment 4 was designed to test a specific explanation for why extinction relied so much on the number of trials, and not trial length, in Experiments 1 to 3 (and in most of the previously published experiments on this topic: Drew et al., 2004; Golkar et al., 2013; Haselgrove & Pearce, 2003). During conditioning in those first three experiments, reinforcement with food always coincided precisely with termination of the CS. As a result, the rats may have specifically learned an association between CS offset and food. Therefore, the response rates we recorded during each CS presentation may have been based either on a second order association between the CS and its offset, or on generalization between features of the CS offset and features of the ongoing CS. In either case, the loss of responding during extinction training may have depended on extinction of the association between CS offset and food, and the opportunity to extinguish the offset-food association would only occur once per trial and would be independent of the length of the trial. To address this possibility, the conditioning phase in Experiment 4 involved delivering food at a variable time within each CS presentation, well before and uncorrelated with the CS offset. Despite these measures to prevent the rats from learning an association between CS offset and food, Experiment 4 replicated the result obtained in the previous experiments, showing that extinction was once again unaffected by large differences in trial length.

Our findings are largely consistent with the results reported previously by Haselgrove and Pearce (2003), Drew et al. (2004), and Golkar et al. (2013). Those authors also reported that the development of extinction was affected by the number of extinction trials and did not depend on total duration of CS exposure during extinction. Those studies used between-subjects designs, whereas the present experiments used within-subjects designs. Therefore, the

fact that extinction did not depend on total duration cannot be due to limitations specific to one or other design (e.g., problems with unequal baseline response rates or high inter-subject variability that can affect results in between-subjects designs, or differences between conditions in within-subjects designs being obscured by generalization between stimuli given different treatments). Collectively, all of these studies are inconsistent with the report by Shipley (1974) who concluded that extinction is determined entirely by total duration of CS exposure. The discrepancy cannot be put down to differences between appetitive and aversive conditioning because both Shipley's experiment and the experiment by Golkar et al. studied extinction of conditioned fear to a CS paired with shock. However, there are characteristics of Shipley's experiment that make us cautious in generalizing his findings beyond the procedures he used (see also Drew et al., 2004, for discussion of Shipley's procedure). In his procedure, rats were given 20 conditioning trials in a single session, which was immediately followed by the single session of extinction. The test for residual fear was then conducted within an hour of the extinction session. There is evidence that extinction training, when conducted immediately after conditioning, can have atypical characteristics (Maren, 2014). For example, immediate extinction can produce more rapid but less durable response loss (Woods & Bouton, 2008). Further, massing multiple extinction trials into a single session, as Shipley did, can effect a short-term reduction of fear, but may not produce longer-term extinction (Li & Westbrook, 2008). Finally, there is evidence in Shipley's own data that the relatively short time between conditioning and test might also have impacted on his results. Among two groups that were not given any extinction treatment, a group given 20 pairings of the 100-s CS with shock did not show evidence of conditioned fear when tested later that day, but did show fear on a re-test

the following day; whereas a group given 20 pairings of the 20-s CS with shock showed fear on the first test but none on the re-test the following day. Given these issues, and the discrepancy between Shipley's results and all four subsequent studies, it would be prudent to regard his results with caution until they are replicated using procedures in which conditioning, extinction, and test are conducted on separate days, as is more commonly done.

The present experiments consistently found that extinction was unaffected by trial duration. This contrasts with evidence for an effect of trial length in both the results reported by Haselgrove and Pearce (2003) and those reported by Drew et al. (2004). In their first three experiments, Haselgrove and Pearce reported that long CS presentations produced faster extinction than short presentations. In those experiments, the rats were trained with a 10-s CS-US interval, and extinction was more rapid when the CS was presented for 30 or 270 s compared with 10 s. Thus, this effect of trial length was obtained using CS durations that extended well beyond the CS-US interval, which was not the case in any of the experiments we present here. Much of the effect of trial length reported by Haselgrove and Pearce could be attributed to a decline in responding during long CS presentations once the trial exceeded the remembered time of reinforcement. Indeed, Drew et al. found some evidence that long CS presentations during extinction (four times longer than the CS-US interval) were less effective than shorter presentations (equal to the CS-US interval) when, on subsequent test, the CS was only presented for the length of the CS-US interval.

Haselgrove and Pearce (2003) and Drew et al. (2004) also reported that extinction was sensitive to differences in trial length when the lengths were shorter than the trained CS-US

interval. In their last two experiments, Haselgrove and Pearce trained rats using a long CS (60 s) and found that responding extinguished more rapidly when each extinction trial was short (10 s) rather than long (60 s). However, because the groups receiving these different trial lengths were matched for total duration of CS exposure during extinction, the faster extinction in the group with the short CS could be attributed to the much larger number of extinction trials given to that group. Drew et al. also found a difference in extinction between groups given short (4 s) versus long (8 s) extinction trials after conditioning with a fixed 8-s CS-US interval. However, they found that the short trials produced less effective extinction than the long trials when the animals were tested for responding to CS presentations that lasted for 8 s. Thus, in this case, longer trials did produce more effective extinction than short trials, but this result was almost certainly obtained because the subjects had been trained with a fixed 8-s CS-US interval. This training would be expected to reduce the effectiveness of short (4-s) extinction trials because they stopped short of the remembered time of reinforcement. This interpretation does not contradict the present findings, in which CS duration did not influence extinction, because our results were obtained after training on variable CS-US intervals. In our experiments, short and long CS durations were equally often reinforced, which would make them equally effective during extinction.

The fact that extinction is not determined by the total time that the CS has been presented since reinforcement is problematic for information-based models of conditioning, like Rate Estimation Theory (Gallistel & Gibbon, 2000), because these models explicitly predict that extinction should depend on total CS exposure (Gallistel, 2012). Our finding that extinction is not sensitive to differences in CS duration also has important implications for real-time

associative models of conditioning. A relatively simple real-time model, in which the CS-US association is updated continuously, would predict that responding should continue to extinguish across the full length of long extinction trials, even if associability of the CS, and thus rate of extinction, changes as time elapses across the trial (e.g., Wagner, 1981). More recent componential models treat the CS as a cascading sequence of elements, or behavioural states, in which each element forms an independent association with the US (Desmond & Moore, 1988; Grossberg & Schmajuk, 1989; Machado, 1997; Vogel et al., 2003). The variable CS-US intervals we have used in the present experiments should ensure that all relevant CS elements acquire associative strength, but extinction of each element's association will depend on the length of the extinction trial. Short extinction trials would only extinguish the associative strength of the initial CS elements, whereas long extinction trials would extinguish all CS elements. Thus, this type of account does predict a difference in extinction between short and long CS exposures, but that difference should only be evident when comparing responding across long CS presentations. As such, this account predicts that short and long extinction might appear equally effective when responding is measured across a short time window at the start of the CS, as was observed in Experiment 2. However, the account also predicts that long trials should be more effective than short ones when responding is measured across the full length of a long CS presentation. This second prediction is disconfirmed by the results of Experiments 3 and 4.

The current results bear on some of our recent experiments that investigated how the acquisition of responding is affected by the amount of non-reinforced exposure to the CS during conditioning. Those experiments, like the present ones, trained rats with variable CS-US

intervals, and found that responding was lower when the mean duration of the CS during conditioning was longer (Harris & Carpenter, 2011), as replicated here in Experiment 2. Moreover, we found that the acquisition of responding was directly related to the total (cumulative) time of CS exposure between presentations of the US (Harris, Patterson, & Gharaei, 2015). Across a large number of experiments, rats did not discriminate between a short CS that was reinforced on every n^{th} trial and a CS that was n times longer but reinforced on every trial. This suggests that, during conditioning, cumulative non-reinforced exposure to the CS is important, as would be predicted if the CS-US association is continuously extinguishing while the CS is present without the US. This conclusion also predicts that the rate at which responding is lost during extinction training should be sensitive to the cumulative duration of CS exposure. Yet this prediction is disconfirmed by the experiments presented here. Thus, it would seem that non-reinforced exposure to the CS has different effects when incorporated into conditioning compared with its effects during explicit extinction training. Learning about non-reinforcement seems to occur in a continuous fashion during conditioning, but is trial-based during extinction.

In conclusion, we have found that extinction of responding is not determined by the total amount of time that the CS has been presented since reinforcement. Instead, extinction appears to be entirely sensitive to the number of extinction trials, and not at all sensitive to the length of those trials. This latter observation is all-the-more significant because, in all the experiments presented here, rats were trained with variable CS-US intervals so that the likelihood of the US, and thus expectation of reinforcement, was equated at each moment during a trial. Our findings are difficult to reconcile with rate-based models of conditioning

(Gallistel & Gibbon, 2000) but also with real-time associative models which assume that extinction should develop in a more-or-less continuous fashion across the length of a trial (Machado, 1997; Vogel et al., 2003; Wagner, 1981). They are also incompatible with an inhibitory S-R account of extinction in which response loss is proportional to the amount of responding during extinction (Rescorla, 1997). Instead, our results show that extinction, at least with the procedures used here³, is event-based. By this we mean that each CS presentation is treated as a single episode, rather than a continuous experience. As such, a non-reinforced trial during extinction is treated as the absence of a single opportunity for reinforcement rather than a continuous experience of disappointment. An implication of this is that learning during extinction might be sensitive to the trial-based reinforcement rate during conditioning rather than the reinforcement rate per unit time. This would be consistent with reports of a partial reinforcement extinction effect (slower extinction) which compare CSs that have matched cumulative rates of reinforcement but different trial-based rates of reinforcement (Bouton, Woods, & Todd, 2014; Haselgrove, Aydin, & Pearce, 2004).

³ Another caveat is that our experiments have focused on the rate at which responding extinguishes, but there might be aspects of response extinction that could show a differential impact of exposure duration, such as its sensitivity to spontaneous recovery or renewal (Bouton, Westbrook, Corcoran, & Maren, 2006). Such an enquiry is beyond the scope of this study, but would be worth exploring in future work.

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Table 1. Summary of designs of Experiments 1 to 4. Each experiment involved 4 or 5 CSs. The table shows for each stimulus how many trials the CS was presented per session, the length of each presentation, and the percent of trials that were reinforced with food, for both the training and extinction phases. In Experiments 1 to 3, the CSs were variable duration and reinforced at offset. In Experiment 4, the CSs had fixed durations during conditioning and were reinforced at a variable time (VT) during the CS.

Experiment (and Group)	CS	Training	Extinction
Exp 1	A	15 x ~20s 100%	5 x ~20s 0%
	B	15 x ~20s 100%	20 x ~5s 0%
	C	15 x ~20s 100%	20 x ~20s 100%
	D	15 x ~20s 100%	20 x ~20s 100%
Exp 2	A	12 x ~40s 100%	12 x 15s 0%
	B	12 x ~40s 100%	12 x 75s 0%
	C		12 x ~15s 100%
	D		12 x ~75s 100%
	X	12 x ~40s 0%	
Exp 3 Group 1	A	12 x ~15s 100%	12 x 30s 0%
	B	12 x ~15s 100%	30 x 10s / 2 x 30s 0%
	C	12 x ~30s 100%	12 x 60s 0%
	D	12 x ~30s 100%	10 x 15s / 2 x 60s 0%
	X	12 x ~15s 0%	20 x ~15s 100%
Exp 3 Group 2	A	12 x ~30s 100%	12 x 60s 0%
	B	12 x ~30s 100%	30 x 20s / 2 x 60s 0%
	C	12 x ~15s 100%	12 x 30s 0%
	D	12 x ~15s 100%	10 x 7.5s / 2 x 30s 0%
	X	12 x ~15s 0%	20 x ~15s 100%
Exp 4	A	9 x 40s VT20s 100%	8 x 10s / 1 x 40s 0%
	B	9 x 40s VT20s 100%	9 x 40s 0%
	X	9 x 20s VT10s 100%	18 x 20s VT10s 100%
	Y	9 x 20s 0%

Figure 1. Top: Mean response rates during acquisition (Sessions 1-20) and subsequent extinction (Sessions 21-35) for rats in Experiment 1. Rats were trained with four CSs, each reinforced with a food pellet after a mean CS-US interval of 20 s. Responding was then extinguished for two of the CSs, A and B, while the other two (C and D) continued to be reinforced. Responding to A was extinguished using a few long non-reinforced presentations without reinforcement (5 trials per session, each lasting 20 s on average). Responding to B was extinguished using many short presentations without reinforcement (20 trials per session, each lasting 5 s on average). Despite the fact that A and B were matched for total duration of exposure per session (100 s), responding to B extinguished more rapidly than to A. This difference is also evident even when response rates to A are only analysed during the first ¼ of each trial (shown as the black dashed line) corresponding to the trial length for B. **Bottom:** Response rates to A and B during extinction, plotted across consecutive trials (each point is the average of a block of five trials).

Figure 2. Mean response rates during acquisition (Sessions 1-20), extinction (Sessions 21-34), and re-test (Session 35) in Experiment 2. Rats were trained with two CSs, A and B, reinforced after a mean CS-US interval of 40 s. A third CS, X, was presented without reinforcement. In the following phase, responding to A and B was extinguished by presenting each CS without reinforcement 12 times per session. The two CSs differed in the duration of those presentations: A was presented for 15 s each trial, B was presented for 75 s each trial. Responding to B decreased more rapidly than to A. However, this difference was not evident when response rates to B were analysed from only the first 15 s of each trial, corresponding to the length of each presentation of A. Across these extinction sessions, the rats acquired responding to two new CSs, C and D, the were reinforced after an average of 15 s and 75 s, respectively. On Session 35, the rats were again tested for responding to A and B under extinction, but this test occurred immediately after a session in which A was presented 20 times with reinforcement. On the re-test, plotted here, response rates were high for A but remained low for B, demonstrating that the rats did discriminate well between the two CSs.

Figure 3. Response rates during acquisition (left panels) and extinction (right panels) for Group 1 (top panels) and Group 2 (lower panels) in Experiment 3. Both groups were trained with two CSs reinforced after a mean of 15 s (15s+), and two CSs reinforced after a mean of 30 s (30s+). A fifth CS (X) was not reinforced during training. Responding to A, B, C, and D was then extinguished. At this time, X was reinforced so that the rats continued to receive some reinforcement during the extinction phase. During extinction, CSs A and B (black lines) were matched for total duration of exposure per session during extinction, but differed in the number of trials. Responding extinguished more rapidly for the more frequent CS (B) than the less frequent one (A) for both groups of rats. CSs C and D (orange/gray lines) were matched for the number of trials per session, but differed in the total duration of exposure per session. Responding extinguished at the same rate for these two CSs for both groups.

Figure 4. Mean response rate across the 35 sessions in Experiment 4. The rats were trained for 25 sessions with 3 CSs that were reinforced with food at a variable time (VT) during the CS. Two of the CSs, A and B, had a fixed duration of 40 s and a mean reinforcement time of 20 s. The third CS, clicker, had a fixed duration of 10 s and a mean reinforcement time of 10 s. A fourth stimulus, buzzer, was presented without reinforcement throughout the training sessions. From Session 26 to 35, responding to A and B was extinguished by presenting each CS without reinforcement, while the clicker continued to be reinforced as in the previous sessions. During these extinction sessions, all presentations of B were 40-s long, and all-but-one presentation of A were 10-s long. Each session included one 40-s presentation of A. Response rates shown here are from 40-s trials only.