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Interstimulus interval as a discriminative stimulus: Evidence of the generality of a novel asymmetry in temporal discrimination learning

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Abstract

Three appetitive conditioning experiments with rats examined temporal discrimination learning within Pavlovian conditioning trials. In all experiments, the duration of a feature white-noise stimulus signaled whether or not a subsequent 10-s target tone would be reinforced. In Experiment 1, the feature durations were 4 and 1 min. For one group of rats (Group 4+/1–), 4 minutes of noise signaled that the tone would be reinforced and 1 minute of noise signaled that the tone would not be reinforced. A second group (Group 1+/4–) was trained with the reverse contingency. The results showed a clear asymmetry in temporal discrimination learning: Rats trained with 4+/1– (Long+/Short–) learned the discrimination readily (responding more in the tone on reinforced than on nonreinforced trials), whereas rats trained with 1+/4– (Short+/Long) did not. In Experiment 2, the feature durations were shortened to 60 and 15 s. Due to strong excitatory conditioning of the 15-s feature, the reverse asymmetry was observed, with the Short+/Long– discrimination learned more readily than the Long+/Short– discrimination. However, Experiment 3 demonstrated that the original Long+/Short– advantage could be recovered while using 60– and 15-s feature durations if the excitatory conditioning of the feature was reduced by including nonreinforced feature trials. The results support previous research involving the timing of intertrial intervals and are consistent with the temporal elements hypothesis which holds that the passage of time is encoded as a series of hypothetical stimulus elements.

Keywords

Temporal discrimination; timing; interstimulus interval; feature-positive effect

Time plays a fundamental role in both what is learned in Pavlovian conditioning, and in how that learning is expressed. For example, in his discussion of *inhibition of delay*, Pavlov (1927) acknowledged that the temporal relationship between the conditioned stimulus (CS) and unconditioned stimulus (US) directly influences the development of the conditioned response (CR). Modern demonstrations have shown that the acquisition of conditioned responding is sensitive to both trial and intertrial durations (Holland, 2000; Lattal, 1999). In addition, recent studies of appetitive conditioning in rats have shown temporally-specific responding in both excitatory conditioning (Williams, Lawson, Cook, Mather, & Johns,

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2008) and inhibitory conditioning (Williams, Johns, & Brindas, 2008). The importance of time has led some to suggest that the primary process of conditioning relies on learning temporal intervals (Balsam & Gallistel, 2009; Gallistel & Gibbon, 2000; Gibbon & Balsam, 1981; cf. Miller & Barnet, 1993; Savastano & Miller, 1998). The study of temporal processes will undoubtedly contribute to a further understanding of learning as it is represented in Pavlovian conditioning.

Recent experiments in our laboratory have investigated how temporal intervals play the role of discriminative stimuli (Bouton & García-Gutiérrez, 2006; Bouton & Hendrix, in press). Bouton and García-Gutiérrez (2006) reported several experiments in which a brief tone CS was paired with a food pellet US. Each successive tone was separated by either a 16-min or a 4-min intertrial interval (ITI). For one group (16+/4-), the CS was paired with the US when the tone followed a 16-min ITI, but not when the tone followed a 4-min ITI. For a second group (4+/16-), the reverse was true: the tone was paired with food when it followed a 4-min ITI, but not when it followed a 16-min ITI. The main finding was that Group 16+/4- (Long+/Short-) learned the discrimination, responding more to the tone following a 16-min ITI than following a 4-min ITI, while Group 4+/16- (Short+/Long-) did not learn the discrimination. This group responded similarly to the tone regardless of whether it followed a short or long ITI.

This asymmetry in temporal discrimination learning was further studied by Bouton and Hendrix (in press). After ruling out alternative explanations of the effect, these authors suggested that the Long+/Short- asymmetry could be explained by a temporal elements hypothesis in which time is represented as a series of hypothetical stimulus elements (A followed by B, followed by C, etc...). If the short interval is represented as A, and the longer interval involves the addition of B (AB), then the Long+/Short- condition is an AB+/A- discrimination and the Short+/Long- condition is an A+/AB- discrimination. In compound conditioning, these are referred to as “feature positive” (AB+/A-) and “feature-negative” (A+/AB-) discriminations, respectively. Thus, according to the temporal elements hypothesis, the Long+/Short- (AB+/A-) temporal discrimination is easier to learn than the Short+/Long- (A+/AB-) temporal discrimination, because feature-positive discriminations are acquired more rapidly than feature-negative discriminations (Hearst, 1984; Jenkins & Sainsbury, 1970).

The studies described above focused on timing during the ITI, i.e., the interval between successive conditioning trials. It is presently an open question whether a similar asymmetry occurs when organisms time *within*, as opposed to between, such trials. In appetitive conditioning, there is evidence of a Long+/Short- asymmetry when animals time the duration of discrete stimuli rather than ITIs. Kyd, Pearce, Haselgrove, Amin, and Aggleton (2007) reported an experiment in which a long (1.5 s) or short (0.5 s) tone was presented, followed by a 10-s interval. At the end of this interval, the US was presented based on a Long+/Short- or a Short+/Long- contingency. In the Long+/Short- condition, the rats spent a greater amount of time checking the food cup during the 10-s interval following the long stimulus as opposed to following the short stimulus. Although the discrimination was also acquired for the Short+/Long- condition, it did not appear until later in training. A similar pattern was observed when the long and short durations were 12 and 3 s, respectively. These results provide a strong parallel to those of Bouton and García-Gutiérrez (2006) and Bouton and Hendrix (in press).

In contrast, Kehoe and Boesenberg (2002) studied within-trial timing in conditioning of the rabbit's eyeblink (nictitating membrane) response. In their procedure, the time that had elapsed in one stimulus (the feature) signaled whether or not a second embedded stimulus (the target) would be reinforced. In Experiment 1, the feature was a 66-s tone and the target

was a 400-msec flashing houselight. The target was presented after the feature had been present for either 5 or 45 s, each on half the trials. For Group 45+ (Long+/Short-), the target was reinforced when it followed the 45-s duration, but not when it followed the 5-s duration. The reverse was true for Group 5+ (Short+/Long-). In marked contrast to Bouton and García-Gutiérrez (2006), Bouton and Hendrix (in press), and Kyd et al. (2007), the discrimination was acquired earlier and was asymptotically more pronounced in Group 5+. The results of Kehoe and Boesenberg thus suggest that some procedures lead to Short+/Long- discriminations being acquired more readily than Long+/Short- ones.

The goal of the present experiments was to examine these asymmetries in within-trial timing in more detail. We developed an appetitive within-trial procedure that was conceptually similar to the one used by Kehoe and Boesenberg (2002). In it, a short (10-s) target tone was paired with a food pellet US depending on whether it followed presentations of a feature stimulus (a white noise) that was either short or long. In Experiment 1, we compared Long+/Short- discrimination training and Short+/Long- discrimination training in two groups of rats using feature durations of 4 and 1 min. These durations had been used as intertrial intervals in previous experiments and are known to support a Long+/Short- effect (Bouton & Hendrix, in press). In Experiment 2 we asked if the contrasting Short+/Long- bias observed by Kehoe and Boesenberg (2002) occurred when we used substantially shorter feature durations of 60 and 15 s. Experiment 2 failed to find the Long+/Short- asymmetry with these feature durations, and instead found strong excitatory conditioning of the 15-s feature in the Short+/Long- group. Experiment 3 then recovered the Long+/Short- effect with the 15/60-s feature durations by using a procedure that reduced the excitatory conditioning of the 15-s cue.

Experiment 1

Experiment 1 examined whether differential feature durations could serve as the basis of discriminative responding to a target tone in an appetitive conditioning paradigm. In Group 4+/1- (Long+/Short-), a 10-s target tone was paired with a food pellet US when it followed a 4-min white noise feature but not when it followed 1 min of the same white noise. In Group 1+/4- (Short+/Long-), the reverse was true: The target tone was paired with the US when it followed a 1-min white noise feature, but not when it followed 4 min of the noise. By using feature durations of 4 and 1 min, this experiment allowed for a test of the generality of earlier work with ITI discriminative stimuli of the same durations (Bouton & Hendrix, in press). In contrast to the procedure used by Kehoe and Boesenberg (2002), in the present design the noise and tone always ended at the same time, making it directly analogous to the intertrial interval experiments. If animals learn about intervals within stimuli in the same fashion they learn about intertrial intervals (e.g., Bouton & Hendrix, in press), then the Long+/Short- discrimination should be learned more readily than Short+/Long-.

Method

Subjects—The subjects were 16 naive, female Wistar rats obtained from Charles River Laboratories, St. Constant, Quebec. They were about 90 days old at the start of the experiment, and were individually housed with ad lib water. The rats were maintained at 80% of their free-feeding weight via food restriction throughout the experiment.

Apparatus—Two sets of four Skinner boxes in separate rooms in the laboratory served as the conditioning chambers. The first set of boxes measured 26 × 25 × 19 cm (length, width, and height). The back and left walls were made of aluminum; the front, right side wall and ceiling were made of acrylic plastic. The floor consisted of tubular steel bars, 16 mm in diameter and spaced 32 mm from center to center. A 5.5 × 5.5 cm food cup was located in

the front wall, 1 cm above the floor and 1 cm to the left of an operant lever. (The operant lever was inactive throughout the experiment). The second set of boxes measured $32 \times 25 \times 21$ cm; the right and left walls, as well as the ceiling were made of acrylic plastic, and the front and back walls were made of brushed aluminum. A recessed 5×5 cm food magazine was centered on the front wall at floor level. The floor was made of stainless steel grids, 5 mm in diameter and spaced 15 mm apart (center to center). Although the two sets of boxes can provide discriminably different contexts, they were not used in that capacity here.

Both types of boxes were housed in sound attenuation chambers illuminated by two 7.5-W incandescent light bulbs mounted to the ceiling of the chambers. A fan attached to the top of the right wall in each sound attenuation chamber provided 60 dBA of background noise. The white noise (65 dBA) was delivered through a 10-cm speaker mounted to the ceiling of the sound attenuation chamber. The target tone was a 3000-Hz tone (80 dBA) delivered through a second speaker mounted to the back of the sound attenuation chamber. The US was two 45 mg food pellets (Traditional formula, Research Diets, New Brunswick, NJ) delivered 0.2 s apart. Head entry into the food magazine served as the conditioned response. Each head entry caused interruption of a photo-beam and was recorded by the computer with a time-stamp. The apparatus was controlled by computer equipment located in an adjacent room.

Procedure—On the first day, the rats were each assigned to a box and received one session of magazine training, in which a total of 30 pellets were delivered at random times over the course of 25 minutes.

Over the next 24 days, the two groups ($n = 8$) received daily conditioning sessions, each approximately 82 minutes in length. There were 16 conditioning trials in each session. The trials consisted of the presentation of a long or short feature noise that co-terminated with a 10-s tone. The interval between the onset of the noise and the onset of the tone was either 4 min or 1 min, each on half of the trials. For one group (Group 4+/1–), the tone ended with the delivery of the US when it followed a 4-min noise duration, but not when it followed a 1-min noise duration. For a second group (Group 1+/4–), the tone ended in a US when it followed a 1-min noise duration but not when the tone followed a 4-min noise duration. The ITI (tone offset to noise onset) was either 4 or 1 min. The ITI durations were balanced in such a way that they occurred equally often with each feature duration. Each session contained 8 reinforced (R) and 8 nonreinforced (N) trials. During every four sessions, the groups received sessions in which the R and N trials occurred in RRNNRRNNRRNNRRN, NRRRNNRRNNRRNNR, RNRNNRRNNRRNNR, NRNRNNRRNNRRNNR sequences.

Data analysis: The computer recorded and time-stamped each interruption of the food cup photo-beam during the entire session. Of particular interest was the 10-s period during the target tone, the 10-s period during the noise that immediately preceded the tone (“pre-tone”), and the 10-s period at the end of the ITI that preceded the noise feature (“pre-feature”). The measure of responding to the target tone was elevation scores of the form $e = t - p$, where t represents the number of responses recorded during the target tone and p represents the number of responses in the pre-tone period. Elevation scores have been used extensively in appetitive conditioning in an effort to separate CS responding from baseline behavior (e.g., Bouton & García-Gutiérrez, 2006). Elevation scores and number of responses in the pre-tone and pre-feature periods were analyzed with parallel analyses of variance (ANOVAs) using rejection criterion of $p < .05$.

Results

The results are summarized in Figure 1, which shows both elevation scores to the target tone and the number of responses in the pre-tone period for both groups over the 24 sessions. Each panel corresponds to a different group, with Group 4+/1– in the upper panel and Group 1+/4– in the lower panel. As the figure suggests, Group 4+/1– learned the discrimination, while Group 1+/4– did not. That is, Group 4+/1– responded more to the tone on reinforced trials than on nonreinforced trials, whereas Group 1+/4– responded to the tone non-differentially. This asymmetry in discrimination learning was not evident in the pre-tone scores (i.e., responding 4 min and 1 min into the feature), where both groups responded more on reinforced trials than on nonreinforced trials, regardless of the feature duration.

Elevation scores—Elevation scores were analyzed with a 2 (Group: 4+/1– vs. 1+/4–) $\times$ 2 (Stimulus: reinforced vs. nonreinforced) $\times$ 24 (Session) ANOVA. This analysis yielded a main effect of Session, $F(23, 322) = 5.98$, and a main effect of Stimulus, $F(1, 14) = 9.30$. There was also a Session $\times$ Stimulus interaction, $F(23, 322) = 1.67$. Most important, there was a significant Group $\times$ Stimulus interaction, $F(1, 14) = 14.00$, consistent with a larger difference in responding on the reinforced and nonreinforced trials in Group 4+/1– as opposed to Group 1+/4–. Thus, whether the animals responded differentially to the tone on reinforced or nonreinforced trials depended on whether the short or long feature was reinforced. No other main effects or interactions were significant.

Elevation scores were also analyzed within each level of Group. For Group 4+/1–, a 2 (Stimulus: reinforced vs. nonreinforced) $\times$ 24 (Session) revealed a main effect of Session, $F(23, 161) = 3.64$, a main effect of Stimulus, $F(1, 7) = 12.87$, and a Session $\times$ Stimulus interaction, $F(23, 161) = 1.79$. Thus, Group 4+/1– acquired the discrimination, responding more on reinforced trials than on nonreinforced trials. In contrast, for Group 1+/4–, an identical ANOVA revealed a main effect of Session, $F(23, 161) = 3.10$, but no effect of Stimulus, or Stimulus $\times$ Session interaction.

Pre-tone scores—The number of responses made in last 10 s of the noise (just prior to the target tone) were analyzed with a 2 (Group: 4+/1– vs. 1+/4–) $\times$ 2 (Stimulus: reinforced vs. nonreinforced) $\times$ 24 (Session) ANOVA. The analysis revealed a main effect of Stimulus, $F(1, 14) = 25.63$, and a Session $\times$ Stimulus interaction, $F(23, 322) = 3.23$. In contrast to the elevation scores, the Group $\times$ Stimulus interaction did not approach significance. Thus, both groups responded more at the end of the feature on their reinforced trials as opposed to nonreinforced trials, and this effect increased over training.

Pre-feature scores—Responding in the last 10 s of the ITI, prior to the onset of both long and short feature cues, was also analyzed. A 2 (Group: 4+/1– vs. 1+/4–) $\times$ 2 (Stimulus: reinforced vs. nonreinforced) $\times$ 24 (Session) ANOVA revealed a main effect of Session, $F(23, 322) = 1.79$. No other main effects or interactions were significant. The mean pre-feature scores were 1.16 and 0.80 for Group 4+/1– and Group 1+/4–, respectively.

Responding in real time—Figure 2 shows head entry rate per second over 5-s bins during the last block of acquisition (sessions 21 to 24). Each panel illustrates responding during the ITI, the feature noise, and the target tone on both reinforced and nonreinforced trials in one group. Responding to the tone is shown in the last 2 points to the right of the solid line. For each group, moving from left to right on the abscissa, the first vertical dashed line corresponds to the onset of the 4-min noise and the second dashed line corresponds to the onset of the 1-min noise. Thirty seconds of ITI responding is shown just prior to the onset of both feature types (to the left of the dashed lines). Group 4+/1– showed anticipatory

behavior from the onset of the 4-min noise. However, it is also clear that the rats elevated their responding more to the tone at the end of reinforced trials (4-min) than on the nonreinforced (1-min) trials. Group 1+/4- showed anticipatory behavior from the onset of the 1-min noise length, but this did not translate into discriminative performance to the tone.

Discussion

Experiment 1 extended previous work on temporal discriminations by replicating the Long+/Short- asymmetry we have found with ITIs (Bouton & García-Gutiérrez, 2006; Bouton & Hendrix, in press) in a method studying within-trial timing. A group given a Long+/Short- discrimination learned to respond differentially to the tone on reinforced and nonreinforced trials, whereas a group given a Short+/Long- discrimination did not. The present experiment demonstrated the Long+/Short- bias with feature durations, showing that the asymmetry we have observed in timing intertrial intervals also occurs with intra-trial timing.

One aspect of the data did not replicate the findings of Bouton and Hendrix (in press). In contrast to the work with ITIs, where responding at the end of 4-min ITIs also demonstrated the Long+/Short- effect, there was no corresponding asymmetry in pre-tone responding at the end of the noise feature. One obvious difference between this procedure and the experiments using ITIs is that the current pre-tone period occurred at the end of a signal for food (the noise) that was itself separated by ITIs. Presumably, the onset of such a stimulus is more salient than an ITI, which might result in stronger excitatory conditioning. Perhaps this helps account for the good, and apparently equivalent, discrimination in the pre-tone period for both Group 4+/1- and Group 1+/4-. Since conditioning is typically stronger with shorter CSs (Bouton & Sunsay, 2003; Holland, 2000; Lattal, 1999), any Long+/Short- asymmetry in the pre-tone period could have been obscured to some extent by superior conditioning with the 1-min noise in Group 1+/4-.

Experiment 2

Although the results of Experiment 1 are consistent with earlier analyses of ITI timing, they are not consistent with the results of Kehoe and Boesenberg (2002) described earlier. In addition to using a different conditioning preparation, Kehoe and Boesenberg used intervals that were considerably shorter in duration (5 s and 45 s) than the ones used in Experiment 1. Our second experiment was therefore designed to begin to approximate the intervals used by Kehoe and Boesenberg. To do this, the 4 and 1-min feature durations were replaced with shorter durations of 60 and 15 s.

Method

Subjects and Apparatus—The subjects were 16 naive, female Wistar rats from the same supplier as those in Experiment 1. The rats were housed and maintained in the same fashion. The apparatus, stimuli, and US were the same as those in Experiment 1.

Procedure—As in Experiment 1, rats received magazine training on the first day of the experiment. In a single training session, a total of 30 pellets were delivered over the course of 25 minutes.

Following magazine training, two groups of rats ($n = 8$) received 24 daily conditioning sessions, each approximately 82 minutes in length. As in Experiment 1, the feature cue was a white noise and the target was a 10-s tone which occurred in the final 10 s of the noise. For one group (Group 60+/15-), the tone was paired with the US when it followed 60 s of noise, but not when the tone followed 15 s of noise. For the second group (Group 15+/60-), the tone was paired with the US when it followed 15 s of noise but not when it followed 60 s of

noise. In order to make the average interval between USs similar to that in Experiment 1, two ITI lengths were used: 240 s (4 min) or 285 s (4 min, 45 s). Each ITI length preceded the 60- or 15-s feature equally often. Each session consisted of 16 trials, 8 reinforced (R) and 8 nonreinforced (N). The trial sequencing over each 4 day period was the same as Experiment 1.

Results

The results of Experiment 2 are depicted in Figure 3, which shows elevation scores to the target tone and the number of responses in the pre-tone period for both groups over 24 sessions. There was considerably more responding in the pre-tone periods (the end of the noise feature) in this experiment. It is clear that although discriminative responding was learned in both groups, it was mainly controlled by conditioning of the feature. Both groups responded more in the pre-tone period on reinforced vs. nonreinforced trials, but this effect was significantly larger for Group 15+/60-.

Elevation scores—Elevation scores, the main measure of responding to the target tone, were analyzed with a 2 (Group: 60+/15- vs. 15+/60-) $\times$ 2 (Stimulus: reinforced vs. nonreinforced) $\times$ 24 (Session) ANOVA. This revealed a main effect of Stimulus, $F(1, 14) = 6.58$, a Session $\times$ Stimulus interaction, $F(23, 322) = 3.81$, and a Session $\times$ Stimulus $\times$ Group interaction, $F(23, 322) = 2.33$. Although the overall Session $\times$ Stimulus $\times$ Group interaction might suggest that Group 15+/60- acquired the discrimination faster, there is in fact no evidence for this; although the pattern over sessions is different in the two groups, both groups began to discriminate at roughly block 4 (sessions 13 to 16). Within-group analysis revealed that both groups first acquired the correct discrimination, responding more on reinforced trials than on nonreinforced trials, during block 4 (sessions 13 to 16), $F_s(1, 7) \geq 6.14$. Within Group 60+/15-, a 2 (Stimulus: reinforced vs. nonreinforced) $\times$ 24 (Session) ANOVA revealed a main effect of Stimulus, $F(1, 7) = 11.58$. In Group 15+/60-, the same ANOVA likewise revealed a significant Session $\times$ Stimulus interaction, $F(23, 161) = 4.15$.

Pre-tone scores—Pre-tone scores were also analyzed with a 2 (Group: 60+/15- vs. 15+/60-) $\times$ 2 (Stimulus: reinforced vs. nonreinforced) $\times$ 24 (Session) ANOVA. This revealed a main effect of Session, $F(23, 322) = 8.40$, Stimulus, $F(1, 14) = 46.59$, and Group, $F(1, 14) = 6.63$. The effect of Stimulus interacted with Group, $F(1, 14) = 8.54$, which is consistent with the figure's suggestion that the discrimination was larger in Group 15+/60-, the Short+/Long- group, with more responding on the reinforced 15-s trials. The effect of Stimulus also interacted with Session, $F(23, 322) = 5.44$.

The pre-tone scores were also analyzed within each group. For Group 60+/15-, there were significant effects of Session, $F(23, 161) = 4.61$, and Stimulus, $F(1, 7) = 16.15$, as well as a Session $\times$ Stimulus interaction, $F(23, 161) = 2.95$. For Group 15+/60, there were also significant effects of Session, $F(23, 161) = 4.79$, and Stimulus, $F(1, 7) = 31.09$, as well as a Session $\times$ Stimulus interaction, $F(23, 161) = 3.28$.

Pre-feature scores—A 2 (Group: 60+/15- vs. 15+/60-) $\times$ 2 (Stimulus: reinforced vs. nonreinforced) $\times$ 24 (Session) ANOVA revealed a main effect of Session, $F(23, 322) = 3.63$, and a Session $\times$ Group interaction, $F(23, 322) = 2.39$. There was also a main effect of Group, $F(1, 14) = 18.85$, indicating that Group 15+/60- responded more in this time bin than Group 60+/15-. Mean responding during this period was 1.10 and 2.38 for Group 60+/15- and Group 15+/60-, respectively. One possible explanation for this difference might be that stronger conditioning of the 15-s feature cue in Group 15+/60- could result in greater second-order conditioning to the context.

Responding in real time—Responding over 5-s bins during the last four sessions of acquisition (sessions 21 to 24) is presented in Figure 4. This figure reinforces the idea that head-entry behavior was controlled primarily by the onset of the noise. Although this was true for both groups, it is evident that the difference between pre-tone responding at the end of long and short trials was much larger in Group 15+/60-. Note also that this group showed a clear temporal discrimination in the noise, such that responding in the 60-s trial peaked at about the moment the tone was presented and reinforced on the positive (15 s) trials and then rapidly declined.

Discussion

In the present experiment, both groups learned to respond more during the tone on reinforced trials than on nonreinforced trials, and there was no clear statistical difference in how rapidly this was learned. However, there was a much different pattern evident in the pre-tone scores, which represent responding at the end of the feature just prior to presentation of the target tone. In the pre-tone period, Group 15+/60- showed a very rapid acquisition of the discrimination, with especially high responding on the reinforced trials, whereas Group 60+/15- developed a more modest discrimination more slowly. By the end of training, the discrimination in the pre-tone scores was much larger in Group 15+/60-. This pattern is reminiscent of the results of Kehoe and Boesenberg (2002), who found better discriminative responding in a Short+/Long- than a Long+/Short- condition. It is worth noting that the present pattern is consistent with research mentioned above indicating that excitatory conditioning is especially strong with short interstimulus intervals (ISIs). That is, the 15-s reinforced trial in Group 15+/60- supported stronger conditioned responding in the feature than did the 60-s reinforced trial in Group 60+/15-. In the case of Kehoe and Boesenberg, a similar scenario could have allowed strong conditioning of the feature in the Short+/Long- group to either elicit eyeblink responding directly or potentiate eyeblink responding to the target (Brandon & Wagner, 1991; see also Wagner & Brandon, 1989, pp. 175–182).

It is also interesting to note that the elevation scores, which were used as the primary measure of responding to the tone, subtract responding prior to the tone from responding during the tone. They indicated very little differential responding controlled by the tone. However, if we simply count the number of head entries made during the tone itself, the pattern in the data is perfectly consistent with Kehoe and Boesenberg (2002). Over the last 4 sessions of conditioning, Group 60+/15- averaged 5.95 responses on reinforced trials and 3.51 responses on nonreinforced trials. Group 15+/60-, however, averaged 8.20 responses on reinforced trials and 2.40 responses on nonreinforced trials. If this result is due at least in part to the fact that short ISIs support better conditioned responding, then reducing the level of conditioning to the noise should eliminate this bias and recover the Long+/Short- asymmetry found in Experiment 1.

Experiment 3

It is clear from Experiment 2 that features of short durations, as used in that experiment, can directly control the timing of the response in this discrimination paradigm. Thus, Experiment 3 was designed to reduce performance elicited by the feature cue. Two groups of rats were trained identically to those in Group 60+/15- and Group 15+/60- from Experiment 2, with one exception. In an effort to reduce conditioning to the noise feature, two nonreinforced presentations of the noise were added to each ITI. According to both trial-based models of conditioning (e.g., Rescorla & Wagner, 1972) and time accumulation models of conditioning (e.g., Gallistel and Gibbon, 2000), the added noise presentations should reduce or delay excitatory conditioning to the noise. If strong excitatory conditioning of the 15-s cue in the 15+/60- group obscured the Long+/Short- effect in Experiment 2,

then the extra nonreinforced trials might allow the Long+/Short− asymmetry observed in Experiment 1 to reappear.

Method

Subjects and Apparatus—The subjects were 16 naive female Wistar rats obtained from the same supplier and housed and maintained in the same fashion as the previous experiments. The apparatus, stimuli, and US were the same.

Procedure—Two groups of rats ($n = 8$) were trained identically to Group 60+/15− and Group 15+/60− in Experiment 2, with one exception: Two nonreinforced presentations of the noise were introduced between successive trials in the Experiment 2 trial schedule. During each ITI (which were 240 or 285 s in duration), one 25-s noise and one 70-s noise were presented (in a counterbalanced sequence over the course of the session). These extra noises were presented alone, without additional tone or US presentations. There was a minimum of 30 s between offset of any noise and the onset of the next noise. Each session consisted of 8 reinforced (R) and 8 nonreinforced (N) noise-tone trials. Because 2 extra nonreinforced noises were added for every trial, each session contained a total of 48 noise presentations and 16 target tone presentations. Eight noise trials contained a tone and were reinforced, 8 noise trials contained a tone and were not reinforced, and 32 noise trials contained no tone and were not reinforced. Overall, half of the noise presentations were 25 s and half were 70 s in duration.

Results

The results are shown in Figure 5. Despite the use of the short feature-target intervals studied in Experiment 2, the results of this experiment were consistent with the findings of Experiment 1. Whereas Group 60+/15− developed more responding during the tone on reinforced as opposed to nonreinforced trials, Group 15+/60− did not fare as well. In fact, this group initially showed a bias towards elevating more to the tone on nonreinforced trials. There was thus a very clear Long+/Short− effect.

Elevation scores—Elevation scores to the target tone were analyzed with a 2 (Group: 60+/15− vs. 15+/60−) $\times$ 2 (Stimulus: reinforced vs. nonreinforced) $\times$ 16 (Session) ANOVA. This revealed a main effect of Session, $F(15, 210) = 9.04$, which interacted with Stimulus, $F(15, 210) = 2.06$. Although the main effect of Stimulus was not significant, the Stimulus $\times$ Group interaction was, $F(1, 14) = 26.76$. In this experiment, discrimination learning with the 60+/15− procedure was superior to that with 15+/60−.

Elevation scores were further analyzed within each group with a 2 (Stimulus: reinforced vs. nonreinforced) $\times$ 16 (Session) ANOVA. For Group 60+/15− the analysis revealed a main effect of Session, $F(15, 105) = 4.56$, Stimulus, $F(1, 7) = 20.12$, and a Session $\times$ Stimulus interaction, $F(15, 105) = 2.42$. For Group 15+/60−, there was a main effect of Session, $F(15, 105) = 5.13$, and a main effect of Stimulus, $F(1, 7) = 7.95$. Notice that for Group 15+/60−, the main effect of Stimulus took the form of (incorrectly) more tone responding in the nonreinforced 60-s trials.

Pre-tone scores—The pre-tone scores were analyzed with a 2 (Group: 60+/15− vs. 15+/60−) $\times$ 2 (Stimulus: reinforced vs. nonreinforced) $\times$ 16 (Session) ANOVA. There was a main effect of Session, $F(15, 210) = 5.84$, of Stimulus, $F(1, 14) = 42.21$, and a significant Stimulus $\times$ Session interaction, $F(15, 210) = 3.82$. The effect of Stimulus also interacted with Group, $F(1, 14) = 4.68$. Group 15+/60− showed better differential responding at the end of the reinforced and nonreinforced noises.

Within Group 60+/15–, a 2 (Stimulus: reinforced vs. nonreinforced) $\times$ 16 (Session) ANOVA revealed a main effect of Session, $F(15, 105) = 2.17$, a main effect of Stimulus, $F(1, 7) = 8.80$, and a Session $\times$ Stimulus interaction, $F(15, 105) = 1.95$. For Group 15+/60– an identical ANOVA revealed a main effect of Session, $F(15, 105) = 4.26$, a main effect of Stimulus, $F(1, 7) = 40.19$, and a Session $\times$ Stimulus interaction, $F(15, 105) = 2.66$.

Pre-feature scores—A 2 (Group: 60+/15– vs. 15+/60–) $\times$ 2 (Stimulus: reinforced vs. nonreinforced) $\times$ 16 (Session) ANOVA revealed a significant main effect of Session, $F(15, 210) = 1.83$. No other main effects or interactions were significant. The mean pre-feature scores were 1.60 for Group 60+/15–, and 1.71 for Group 15+/60–.

Responding in real time—Figure 6 shows responding over 5-s bins during the last block of acquisition (sessions 13 to 16). Group 60+/15– showed some anticipatory head-entry behavior from the onset of the noise. For this group there was a clear discrimination at tone onset. On 60-s noise trials, the rats dramatically increased responding during the tone, peaking just prior to US-delivery. This increase in responding to the tone was greatly attenuated on 15-s noise trials. For Group 15+/60–, although head-entry behavior was elicited by the onset of the noise, elevation to the tone stimulus was similar regardless of the noise duration.

Discussion

Experiment 3 once again demonstrated the Long+/Short– asymmetry in temporal discrimination learning. Rats in Group 60+/15– successfully learned the discrimination, elevating their responding to the tone more following a long trial than following a short trial. Importantly, Group 15+/60– never learned to respond to the tone more on reinforced than nonreinforced trials. The results suggest that the Long+/Short– asymmetry is possible with short-duration feature stimuli provided nonreinforced feature trials are included. These trials reduce the excitatory power of the noise, and make the pattern with 60/15-s discriminations more similar to that observed with 4/1-min discriminations (Experiment 1).

General Discussion

The current experiments provide evidence for asymmetrical temporal discrimination learning when timing occurs within Pavlovian conditioning trials. Consistent with previous research on timing during intertrial intervals (Bouton & García-Gutiérrez, 2006; Bouton & Hendrix, in press), Experiment 1 found that Long+/Short– discriminations are learned more readily than Short+/Long– discriminations. Experiment 1 used feature durations of 4 and 1 min, which have previously been shown to support the Long+/Short– effect when used as ITIs (e.g., Bouton & Hendrix, in press). Experiment 2 then studied relatively short feature durations of 60 and 15 s. Here the Long+/Short– asymmetry was reversed, with a Short+/Long– discrimination appearing to be learned more rapidly. This finding replicated the results of Kehoe and Boesenberg (2002) in our appetitive conditioning preparation. However, the Short+/Long– asymmetry evident in Experiment 2 was not present in responding to the target tone itself; during the tone, the Short+/Long– and Long+/Short– showed roughly equivalent discriminative responding. Instead, the reversed asymmetry was primarily present in responding during the pre-tone period, that is, during the 10-s interval at the end of the feature. Unlike Experiment 1, the feature acquired strong excitatory conditioning in Experiment 2, and this was especially true in Group 15+/60–, obscuring or masking the Long+/Short– effect. Consistent with this hypothesis, Experiment 3 found the Long+/Short– asymmetry again with the same short feature durations when conditioned responding to the feature was reduced. By adding nonreinforced (extinction) presentations of the feature alone, we found, consistent with Experiment 1, that the Long+/Short–

discrimination in responding to the tone was again learned more readily. The results of Experiments 1 and 3 together thus suggest that within-trial Long+/Short– discriminations are easier to learn than Short+/Long– discriminations when the procedure minimizes excitatory conditioning of the feature.

The results of Experiment 2 are directly analogous to the results reported by Kehoe and Boesenberg (2002). Why did the use of the short feature durations result in a Short+/Long– bias? Both the pre-tone scores and the real-time response trajectories strongly suggest that the noise feature, not the target tone, was the primary stimulus that elicited conditioned responding. Further, the strength of conditioning to the feature was greater in the Short+/Long– discrimination group than in the Long+/Short– group. This effect is consistent with the finding that short ISIs allow better conditioning than long ISIs at the same ITI (e.g., Bouton & Sunsay, 2003; Holland, 2000; Lattal, 1999), an outcome that is consistent with several conditioning theories (e.g., Gallistel & Gibbon, 2000; Wagner, 1981). Thus, relatively rapid development of conditioning in the Short+/Long– group would occur because of better excitatory conditioning of the noise with 15-s, as opposed to 60-s, ISIs.

A less direct but related mechanism might be especially relevant to the nictitating membrane response (NMR) method used by Kehoe and Boesenberg (2002). According to the “affective extension” of Wagner’s SOP model, AESOP (Wagner & Brandon, 1989), a CS can enter into associations with both the sensory and the affective or emotive aspects of the US. Association of the CS with the sensory aspects of the US will allow the CS to elicit a discrete conditioned response, such as an eyeblink. Association of the CS with the emotive aspects of the US will allow the CS to elicit preparatory or emotional responding, such as fear. The emotional conditioned response is predicted to potentiate the sensory eyeblink response that is elicited by a separate target CS (Brandon & Wagner, 1991). Thus, in the case of Kehoe and Boesenberg (2002), fear conditioned to the shorter feature cue could have potentiated eyeblink responding to the target stimulus—even if the 5-s ISI was itself too long to support the eyeblink response (VanDercar & Schneiderman, 1967).

The Short+/Long– asymmetry in Experiment 2 and in Kehoe and Boesenberg (2002) might have also occurred for still another reason. In the eyelid response preparation, it is difficult to acquire a CR to the onset of a stimulus at ISIs beyond a few seconds. However, if two stimuli are presented in sequence, the acquisition of the CR to the onset of the first stimulus can be extended over longer ISIs (Kehoe, Marshall-Goodell, & Gormezano, 1987; Kehoe, Palmer, Weidemann, & Macrae, 2000). Thus, in Kehoe and Boesenberg’s Short+/Long– condition, second-order conditioning to the onset of the feature cue may have summated with excitation to the target cue, leading to high levels of responding on Short+ trials. This would allow the Short+/Long– discrimination to be acquired readily. It is possible that the results of the present Experiment 2 depended on a similar mechanism. In an unpublished experiment, we found no discriminative advantage in a 15+/60– discrimination compared with a 60+/15– discrimination when the target tone was not included in the conditioning procedure; rats in the two groups discriminated reinforced and nonreinforced trials equivalently. Thus, the stronger pre-tone responding seen at 15 s in the 15+/60– procedure depends on the presentation of tones. Whatever the cause of strong Short+/Long– bias in Experiment 2’s 15+/60– procedure, the results of Experiment 3 suggest that reducing the associative strength of the noise feature by adding extra nonreinforced trials allows the opposite Long+/Short– asymmetry to be observed.

The Long+/Short– asymmetry observed in Experiments 1 and 3 is consistent with the temporal elements hypothesis. This view holds that the passage of time, either within or between conditioning trials, involves exposure to a set of hypothetical stimulus elements. For example, the onset of a stimulus may set in motion the activation of sequential and

overlapping stimulus elements at successive time steps (e.g. Desmond & Moore, 1988). Associative strength would accrue to these “temporal elements” following the rules that apply to discrete stimuli (e.g., Rescorla & Wagner, 1972). In the current experiments, Short feature durations might be represented by stimulus element A, while Long features might be represented by the addition of stimulus element B (AB). By allowing the temporal elements to enter into associations with the US based on a competitive learning rule (e.g., Rescorla & Wagner, 1972), the Long+/Short– asymmetry is predicted because that discrimination (AB +/A–) is analogous to a feature-positive discrimination (FP) while the Short+/Long– (A+/AB–) discrimination is analogous to a feature-negative discrimination (FN). Importantly, there is clear evidence that feature-positive discriminations are acquired more readily than feature-negative discriminations (e.g., Bouton & Doyle-Burr, submitted; Hearst, 1984). The feature-positive effect is consistent with elemental models of conditioning (e.g., Rescorla & Wagner, 1972), which suggest that early in training, AB+ (in FP) trials will accrue more associative strength than A+ (in FN) trials. This occurs because on AB+ trials, both A and B enter into a separate association with the US, and the associative strength of both A and B is then summed on subsequent trials. Bouton and Doyle-Burr (submitted) have identified two other reasons why FP discriminations are easier to acquire than FN discriminations. First, there is less generalization of excitation from compound (AB+) to element (A–) in the FP procedure than from element (A+) to compound (AB–) in the FN procedure (e.g., Rescorla & Wagner, 1972). Second, there is also less generalization of extinction from element (A–) to compound (AB+) in the feature-positive procedure than from compound (AB–) to element (A+) in the feature-negative procedure. According to the temporal elements view, these principles of compound conditioning account for the Long+/Short– asymmetry observed in Experiments 1 and 3.

Why did strong excitatory conditioning to the feature obscure the Long+/Short– effect with shorter feature durations in Experiment 2? One possibility is that the onset of the noise presents a very salient cue, **X**, which then decreases in intensity over time (and might become small or negligible after 60 s; cf. Darby & Pearce, 1995). (In SOP theory [Wagner, 1981], elements of the CS node would transition from a focally active state, A1, to the less active state of A2.) When paired with the US, the very salient **X** cue present at the 15-s ISI would gain a great amount of excitation that would allow differential responding to develop in Experiment 2's Short+/Long– discrimination (now **XA**+/AB–). The main result of adding nonreinforced trials with the noise in Experiment 3 would be to weaken the excitatory strength of **X**, thus requiring normal (and slow) FN learning to occur. Whatever the final explanation of this aspect of the results may be, the main points of the present experiments are that (1.) in the absence of strong excitatory conditioning to the feature, Long+/Short– temporal discriminations are easier to learn than Short+/Long– temporal discriminations when timing occurs within conditioning trials, and (2.) that this asymmetry may be most consistent with a temporal elements analysis of time and timing within conditioning trials.

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Figure 1.

Responding in the tone (elevation scores) and in the pre-tone period (“pre”, in no. of responses) over 24 sessions of Experiment 1. Group 4+/1- and Group 1+/4- are shown in separate panels. 4 and 1 refer to duration of the noise-tone interval, while “+” and “-” indicate that the tone was reinforced or nonreinforced, respectively.

Figure 2.

Momentary levels of head entry responding over the final block of conditioning (sessions 21 to 24). Head entries per second are averaged over 5-s time bins and plotted relative to tone (0) onset. Responding during the tone is presented to the right of the solid vertical line. Scheduled US delivery is indicated by the rightmost dashed vertical line. Groups are depicted in separate panels. Group 4+/1- is shown in the top panel and Group 1+/4- is shown in the bottom panel. Short and long feature durations are presented for both groups. Onset of long features begins at -240-s (4 min, dashed vertical line) and onset of short trials begins at -60-s (1 min, dashed vertical line). Responding over the final 60 s of the ITI is also shown for each trial type (for 4-min trials ITI is -300 s to -240 s; for 1-min trials ITI is -120 s to -60 s).

Figure 3.

Responding in the tone (elevation scores) and in the pre-tone period (“pre”, in no. of responses) over 24 sessions of Experiment 2. Group 60+/15– is presented in the top panel and Group 15+/60– is presented in the bottom panel. 60 and 15 refer to the duration of the noise-tone interval, while “+” and “–” indicate that the tone was reinforced or nonreinforced, respectively.

Figure 4.

Momentary levels of head entry responding over the final block of conditioning (sessions 21 to 24). Head entries per second are averaged over 5-s time bins and plotted relative to tone onset. Responding during the 10-s tone is presented to the right of the solid vertical line. Scheduled US delivery is indicated by the rightmost dashed vertical line. Group 60+/15- is shown in the top panel and Group 15+/60- is shown in the bottom panel. Short and long features are depicted for both groups. Onset of long trials begins at -60-s (dashed vertical line) and onset of short trials begins at -15-s (dashed vertical line). Responding over the final 240 s of the ITI is also shown for each trial type (for 60-s trials the ITI is -300 s to -60 s; for 15-s trials the ITI is -255 s to -15 s).

Figure 5. Responding in the tone (elevation scores) and in the pre-tone period (“pre”, in no. of responses) over 16 sessions of Experiment 3. Group 60+/15- is presented in the top panel and Group 15+/60- is presented in the bottom panel. 60 and 15 refer to the duration of the noise-tone interval, while “+” and “-” indicate that the tone was reinforced or nonreinforced, respectively.

Figure 6.

Momentary levels of head entry responding over the final block of conditioning (sessions 13 to 16). Head entries per second are averaged over 5-s time bins and plotted relative to tone (0) onset. Responding during the tone is presented to the right of the solid vertical line. Scheduled US delivery is indicated by the rightmost dashed vertical line. Group 60+/15 is shown in the top panel and Group 15+/60- is shown in the bottom panel. Short and long trials are depicted for both groups. Onset of long features begins at -60-s (dashed vertical line) and onset of short trials begins at -15-s (dashed vertical line). Responding over the final 30 s of the ITI is also shown for each trial type (for 60-s trials the ITI extends 6 data points to the left of -60 s; for 15-s trials the ITI extends 6 data points to the left of -15 s).