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Pavlovian Biconditional Discrimination Learning in the C57BL/6J Mouse

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Abstract

Four experiments using mice examined acquisition of Pavlovian biconditional discriminations in which two stimulus compounds were paired with food (AX+ and BY+) and two were not (AY– and BX–). Temporally asynchronous compounds were generated by using contextual stimuli (Experiment 1) and 15-sec discrete visual cues (Experiments 2A, 2B and 3) to disambiguate when embedded noise or tone stimuli would be paired with food. When food pellets followed both reinforced compounds, successful acquisition was obtained in Experiment 1 but not in Experiments 2A and 2B even though the order of trials was modeled after that used in Experiment 1. However, when differential outcomes followed the reinforced compounds in Experiment 3, acquisition was obtained with discrete cue stimulus compounds. The implications of these results for modulatory models of conditional discrimination learning in animals are discussed.

Keywords

biconditional discrimination; contexts; differential outcomes; mice; modulation; Pavlovian conditioning

1. Introduction

In a conventional Pavlovian biconditional discrimination, four stimuli (A, B, X, and Y) are presented in pairs and correlated with reinforcement and nonreinforcement in such a way that the compounds but not the elements reliably signal trial outcomes. Thus, an unconditioned stimulus (US) may follow the compounds AX and BY but not the compounds AY and BX. Acquisition of a Pavlovian biconditional discrimination is evidenced by greater conditioned responding to the reinforced compounds (AX+ and BY+) than to the nonreinforced compounds (AY– and BX–). The ability of animals to solve this type of discrimination has intrigued learning theorists because of the challenge that successful performance poses for simple but powerful elemental theories of associative learning (e.g., Rescorla & Wagner, 1972). Such models predict no differential responding between the reinforced and nonreinforced compounds: The associative strengths of these compounds should be equal because their individual elements share an identical history of reinforcement and nonreinforcement.

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Pavlovian biconditional discrimination learning has now been demonstrated in a variety of species including humans (Harris & Livesey, 2008; Lober & Lachnit, 2000), rats (Harris et al., 2008; Honey & Watt, 1998, 1999; Wilson & Pearce, 1989), bees (Schubert et al., 2002), rabbits (Saavedra, 1975), and macaque monkeys (Bussey et al., 2002). Several explanations have emerged to explain successful biconditional discrimination learning. Some preserve the fundamental principles of elemental theory (Harris, 2006; McLaren & Mackintosh, 2000, 2002; Rescorla, 1972; Wagner, 2003) whereas others adopt either a completely non-elemental or configural approach (Pearce, 1987, 1994, 2002) or a flexible mix of the two (Melchers et al., 2008).

Our interest in Pavlovian biconditional discrimination learning stems in part from a previous report from our laboratory of a failure to detect Pavlovian biconditional discrimination learning in the C57BL/6J mouse (Fetsko et al., 2005). To our knowledge, this is the only study in the literature that has tried to demonstrate Pavlovian biconditional discrimination learning in mice. Using a discrete cue biconditional discrimination procedure, Fetsko et al. (2005) presented mice with four binary compounds made up of auditory stimuli, either a white noise (N) or tone (T), embedded in the last 5 seconds of 15-sec visual stimuli, either an overhead houselight (L1) or a panel light (L2). Food delivery coincided with the offset of two of the four stimulus compounds (L1-N+, L2-T+); the other two stimulus compounds were nonreinforced (L1-T-, L2-N-). Even after 80 daily training sessions, there was no evidence of differential responding. The rate of head entries into the food magazine during the last 5 sec of the compounds was indistinguishable on reinforced and nonreinforced trials. Given the theoretical ramifications of successful biconditional discrimination learning for both brain and behavioral models of complex discrimination learning (Aggleton et al., 2007; Bussey et al., 2002; Haddon & Killcross, 2006) and the potential value of the mouse as an animal model for studying genes and behavior (Havekes & Abel, 2009; Tecott, 2003), we conducted a series of experiments to examine if Pavlovian biconditional discrimination learning could be obtained in the mouse.

Fetsko et al. (2005) identified three factors that might facilitate acquisition of this type of discrimination. Specifically, they proposed that the properties of the stimuli, the order of trials, and the identity of the food outcomes might be manipulated to reduce task difficulty. Each of these variables was examined separately in the series of experiments reported here. In Experiment 1, mice were trained on a biconditional discrimination with contextual stimuli. In Experiments 2A and 2B, mice were trained on discrete cue biconditional discriminations with a trial order modeled after that of a contextual biconditional discrimination. Finally, in Experiment 3, mice were trained on a discrete cue biconditional discrimination with differential outcomes. Each of these manipulations was expected to yield evidence of Pavlovian biconditional discrimination learning in mice.

2. Experiment 1

Acquisition of a Pavlovian biconditional discrimination was examined in Experiment 1 using contextual cues to disambiguate which one of two discrete auditory cues was followed by a food US. Honey and Watt (1999) showed that a contextual biconditional discrimination was easier to solve than the standard discrete cue biconditional task. In Experiment 2 of their study, rats received concurrent training on two biconditional discriminations using the same pair of auditory stimuli. In two daily sessions, distinct contexts (dotted versus checkered wall liners) signaled when an auditory stimulus would be paired with a food US; in the other daily session conducted in an unlined chamber, two discrete visual cues (constant versus flashing light) signaled when an auditory stimulus would be paired with the same food US. A within-subjects analysis revealed superior acquisition of the contextual biconditional

discrimination. Given these results, we anticipated that using contextual cues might also make it easier for mice to solve a Pavlovian biconditional discrimination.

The basic design of Experiment 1 is outlined in Table 1. The two contexts (A and B) were differentiated by the construction of their respective floors. For Context A, the floor was a smooth sheet of Plexiglas, and for Context B, the floor was composed of stainless steel rods. For all mice, one auditory cue (e.g. a tone, T) was reinforced in Context A and the other auditory cue (e.g., a noise, N) was not. These reinforcement contingencies were switched in Context B. On reinforced trials in both contexts, the same food US was delivered coincident with the offset of the auditory stimulus. Head entries into the food magazine were recorded during the auditory stimuli. The question of interest was whether responding would be greater to the reinforced cue than to the nonreinforced cue in each context.

Following initial training, two assessments were run to determine if the designated contextual cues (different floors) had acquired differential control over responding. In the first series of tests, biconditional performance was examined when all training occurred in the same physical chamber both with and without the Plexiglas floor inserts. During initial training, one set of four boxes was designated Context A and another set of four boxes was designated Context B. Examination of response patterns in these test sessions would reveal if the different floor cues had in fact acquired control over discriminative responding. In the second series of tests, mice were tested in extinction. A feature of contextual biconditional discrimination training that makes it different from standard biconditional discrimination training is the blocking of trials into two discrete and sequential sets in which one cue is reinforced and the other is not. With this arrangement, an animal could use a response rule within each session based on which cue is paired with food at the start of that session. Testing in extinction eliminated food presentations and thus prevented deployment of such a rule. It was expected that if the designated contextual cues controlled biconditional performance, differential responding would remain intact during these extinction tests.

2.1. Methods

2.1.1. Subjects—The subjects were 16 experimentally naïve male C57BL/6J mice from a colony in the Department of Psychology of Brown University. They were approximately 50 days old at the start of the experiment. The mice were housed in pairs in plastic tubs ($27 \times 16.5 \times 12.5$ cm) in a temperature-controlled facility with a 12:12-h light:dark cycle. The tail of one mouse in each pair was marked with a black stripe to distinguish between the individuals housed together. Mice were given enough daily food to maintain them at 85% of their free-feeding weights. Water was always available in the home cage.

2.1.2. Apparatus—The apparatus consisted of eight identical mouse conditioning boxes (Med Associates, St. Albans, VT) measuring $24.0 \times 20.1 \times 18.6$ cm. The two end walls of each chamber were composed of aluminum panels; the side walls and ceiling were clear polycarbonate. Each chamber had a recessed food magazine in the center of one end wall. Operation of the pellet dispenser allowed a single 20-mg food pellet (Formula A, P. J. Noyes Co.) to drop onto the floor of the magazine. An infrared detector and emitter system was mounted on the inside walls of the magazine, permitting automatic recording of head entries into the magazine.

Two distinctive contexts were created by varying the properties of the floor of the chamber. In four boxes (Context B), the floor of each chamber was composed of 0.4 cm stainless steel rods spaced 0.6 cm apart. In the other four boxes (Context A), this grid floor was concealed under a Plexiglas insert measuring $21.0 \times 17.8 \times 0.6$ cm. This Plexiglas insert was locked in place by an $18.4 \times 2.5 \times 0.3$ cm Plexiglas wedge.

Each conditioning chamber was enclosed in a sound-attenuating and light-resistant shell, equipped with a 1-cm spy hole in the front door for observation. A houselight with diffuser (Med Associates, St. Albans, VT) was mounted on the ceiling of the shell directly above the chamber. A 28-V yellow stimulus panel light was mounted 2 cm above the grid floor on the back polycarbonate wall and 3 cm from the right aluminum wall that supported the recessed food magazine. These lights were not used in Experiment 1. Two speakers mounted vertically on the wall opposite the food magazine permitted presentation of a white noise (N) and a 2900 Hz tone (T). Experimental events were controlled and recorded automatically by interfacing (Med Associates, St. Albans, VT) and a 486 computer located in a room adjacent to the mouse conditioning equipment.

2.1.3. Procedure

2.1.3.1. Magazine training: Subjects were given two sessions of magazine training, one in Context A and one in Context B. In each session, 15 food pellets were delivered on a variable-time (VT) 60-s schedule. For eight subjects, the first session was in Context A and the second session was in Context B; for the other eight subjects, this order was reversed.

2.1.3.2. Contextual biconditional discrimination training: There were 36 days of contextual biconditional discrimination training. On each day, subjects received 8 trials with each auditory stimulus in Context A and 8 trials with each auditory stimulus in Context B. For eight subjects, 10-sec presentations of N were immediately followed by food pellets and 10-sec presentations of T were nonreinforced in Context A (A: N+, T-); these contingencies were switched in Context B (B: T+, N-). For the other eight subjects, T was reinforced and N was not in Context A (A: T+, N-); these contingencies were switched in Context B (B: N+, T-). The order in which context training occurred each day was determined by an ABBABAAB Gellerman sequence and counterbalanced across animals and contingencies. An equal number of reinforced and nonreinforced trials occurred in the first half of a session and the order was randomized across days. The order of trials in the second half of a session was the mirror image of the order during the first half of that session. The mean intertrial interval (ITI) was 150 sec. The number of photobeam interruptions by head entry into the magazine was recorded during each 10-sec presentation of an auditory stimulus, the 10 seconds immediately preceding the onset of an auditory stimulus, and during the ITI. Responding in the presence of the auditory stimuli was compared between reinforced and nonreinforced trials to assess discrimination learning.

2.1.3.3. Assessments: In the preceding biconditional discrimination training sessions, four boxes with Plexiglas floor inserts were designated as Context A and four other boxes with stainless steel grid floors were designated as Context B. To demonstrate that the different floors used to distinguish the contexts acquired control over biconditional responding, mice received two training sessions in the box that had always served as Context A and two sessions in the box that had always served as Context B. In one of the two sessions in each box, the grid floor was exposed and in the other session the Plexiglas insert covered the grid floor. Thus, for two sessions, the floors were located in the same boxes used for original training (Congruent); in the other two sessions, the floors were switched (Incongruent) as illustrated in Table 1. Between the two consecutive sessions in the same physical box, mice were removed from the testing chamber, briefly returned to the home cage while the Plexiglas floor was inserted or removed, and then placed back into the test chamber. All other procedural aspects of these two pairs of sessions were identical to the original sessions of contextual biconditional discrimination training.

The second assessment evaluated the effect of testing in extinction. Following completion of the first assessment, mice received three additional days of contextual biconditional

discrimination training. The next day, all mice were tested in extinction, once in Context A and once in Context B. Each extinction session had 4 trials of each auditory stimulus but all trials were nonreinforced. The mean ITI was 150 sec and the trial order was NTTNTNNT. The order of testing in the two contexts was counterbalanced across subjects. If the mice were responding during a training session based on which stimulus was reinforced at the start of that session, differential responding would not be expected in either context during testing.

2.2. Results and Discussion

2.2.1. Contextual biconditional discrimination training—Acquisition of the contextual biconditional discrimination is shown in Figure 1. Mean responses per minute to the reinforced and nonreinforced auditory cues, combined across contexts, are plotted separately in 4-day blocks. Following an initial steep rise and then a gradual decline in responding on both reinforced and nonreinforced trials, robust differential responding emerged as responses to the reinforced cue increased and those to the nonreinforced cue continued to decline. A repeated measures ANOVA of the final block of training revealed a significant main effect of Reinforcement, $F(1,15) = 12.27$, $p < .01$, indicating that the rate of responding was higher on reinforced than on nonreinforced trials. There was no main effect of Context ($F < 1$) and no significant Reinforcement $\times$ Context interaction ($F < 1$). A follow up analysis revealed that the magnitude of differential responding to the reinforced and nonreinforced cues did not differ significantly between the first half (9.8 and 3.1 responses per min) and the second half (9.3 and 2.4 responses per min) of the sessions in the final block of training ($F < 1$).

2.2.2. Assessments—The mean rates of responding on reinforced and nonreinforced trials during the first assessment are shown in Figure 2A. In both the Congruent and Incongruent conditions, there was more responding on reinforced than on nonreinforced trials. Although this difference was numerically larger in the Congruent condition during which the different floor cues were in the same boxes used for original training, it was not statistically significant. A repeated measures ANOVA revealed a significant main effect of Reinforcement, $F(1,15) = 12.07$, $p < .01$, but no significant Reinforcement $\times$ Congruency interaction ($F < 1$), and no main effect of Congruency ($F < 1$). These results confirm that the different floors were effective contextual stimuli and had acquired differential control over responding.

The results of the second assessment, testing in extinction, are shown in Figure 2B. Mean responses per min on reinforced and nonreinforced trials are shown separately for the last session of retraining and for the extinction test. Response rates were higher on reinforced than on nonreinforced trials and this difference was unaffected by the omission of food on reinforced trials during extinction testing. A repeated measures ANOVA revealed a main effect of Reinforcement, $F(1,15) = 9.01$, $p < .01$, but no main effect of Day ($F < 1$) or Reinforcement $\times$ Day interaction, $F(1,15) = 1.18$, $p > .10$.

The results of Experiment 1 demonstrate acquisition of a contextual biconditional discrimination in mice. Differential responding to the reinforced and nonreinforced compounds emerged across training sessions, was stable across the two halves of a session during the last training block, and persisted during extinction testing when food was withheld on reinforced trials. The results of extinction testing suggest that it is highly unlikely that trial outcomes at the beginning of a session were used to guide responding. Collectively, our results show that responding to the auditory cues was controlled by the contexts. To our knowledge, this study provides the first demonstration of successful acquisition of a Pavlovian biconditional discrimination in mice.

3. Experiment 2A

The goal of Experiment 2A was to examine the role of trial order in mediating the discrepancy between successful acquisition of a contextual biconditional discrimination in Experiment 1 and the failure to find evidence of biconditional discrimination learning with discrete cues in Experiment 5 of Fetsko et al. (2005). In contextual biconditional discriminations, it is routine to partition daily training into two sequential sessions such that subjects are exposed to one pair of reinforcement contingencies in one context (e.g., A: X+, Y-) and then the reverse reinforcement contingencies in the other context (e.g., B: Y+, X-), as they were in Experiment 1. In contrast, all four trials types are intermixed in a single session in the conventional discrete cue biconditional discrimination (e.g., AX+, AY-, BY+, BX-), as they were in Experiment 5 of Fetsko et al. (2005).

The impact of segregating trials in this way was examined by Thomas and Goldberg (1985). Three groups of pigeons were trained on a wavelength discrimination in one context and its reversal in a different context. For example, in one context (housetlight and tone), a 538-nm stimulus (S+) was paired with food and a 576-nm stimulus (S-) was not; in a second context (dark and noise), these contingencies were reversed (576-nm S+, 538-nm S-). Group 1 was trained for four days in one context and then for four days in the second context. Group 2 was also trained for four days in each context but the training alternated daily between the two contexts. Group 3 was trained for a total of eight days as well but the contexts alternated minute by minute. Wavelength generalization gradients were then obtained in extinction in both contexts. Stronger conditional control by contexts was found in Groups 1 and 2 relative to Group 3. In fact, only three of the eight pigeons in Group 3 developed gradients that peaked at the appropriate S+ value in each context. Thomas and Goldberg (1985) concluded that the optimal conditions for acquisition of a conditional discrimination balanced opportunities for repeated stimulus comparisons against interference between conflicting memories. Experiment 2A examined if acquisition of a discrete cue biconditional discrimination might therefore be facilitated by packaging the trials in sequential blocks as there were in Experiment 1; for example, AX+ and AY- in one half of a daily session and BY+ and BX- in the other half of a daily session.

Experiment 2A used the same discrete cue biconditional discrimination procedure as Fetsko et al. (2005, Experiment 5) but with a modified trial order. Four compounds were thus created by embedding presentations of an auditory stimulus (tone or white noise) in the last 5 sec of a 15-sec visual stimulus (overhead light or panel light). However, the first half of each daily session contained one reinforced and one nonreinforced compound using one of the lights (e.g., L1-N+ and L1-T-) whereas the second half of each session contained one reinforced and one nonreinforced compound using the other light (e.g., L2-T+ and L2-N-). Acquisition was measured by comparing responding to the embedded auditory cues on reinforced and nonreinforced trials.

3.1. Methods

3.1.1. Subjects—Sixteen experimentally naïve male C57BL/6J mice, approximately 50 days old at the start of the experiment, served as subjects. The conditions for housing and maintenance were the same as those in Experiment 1.

3.1.2. Apparatus—The apparatus was the same as that used in Experiment 1 except that the Plexiglas floor inserts were not used.

3.1.3. Procedure

3.1.3.1. Magazine training: Subjects were given two sessions of magazine training in which 15 food pellets were delivered on a VT 60-sec schedule.

3.1.3.2. Biconditional discrimination training: There were 60 sessions of Pavlovian biconditional discrimination training. Each session consisted of eight 5-sec presentations of each auditory stimuli, noise (N) and tone (T), with a mean ITI of 150 sec. Four presentations of each auditory stimulus were embedded in the last 5 sec of a 15-sec houselight (L1), whereas the other four presentations of each auditory stimulus were embedded in the last 5 sec of a 15-sec panel light (L2). To mimic the packaging of trials in Experiment 1, the trials were sequenced such that the first 8 trials consisted of presentations of each auditory stimulus in the presence of only one light, whereas the last 8 trials trained the same auditory stimuli in the presence of the other light. The order of which light was trained first followed a Gellerman sequence (ABBABAAB). Eight subjects received reinforced presentations of N and nonreinforced presentations of T during L1 (L1-N+, L1-T-) and reinforced presentations of T and nonreinforced presentations of N during L2 (L2-T+, L2-N-). These contingencies were switched for the other eight subjects (L1-N-, L1-T+, L2-N+, L2-T-). The offset of all reinforced stimulus compounds coincided with the delivery of a single food pellet. In this and all subsequent experiments, the number of photobeam interruptions by head entry into the magazine was recorded during each 5-sec presentation of an auditory stimulus, the 10 sec prior to the auditory stimulus when lights were presented alone, and during the ITI.

3.2. Results and Discussion

3.2.1. Biconditional discrimination training—Figure 3 displays responding during the auditory cues on reinforced and nonreinforced trials in blocks of four daily sessions. Response rates gradually increased on both reinforced and nonreinforced trials, reaching a common and stable asymptote by the fifth block of training. At the end of 60 days of training, there was no evidence of differential responding between reinforced and nonreinforced trials. A repeated measures ANOVA on the final block of four sessions found no significant main effect of Reinforcement ($F < 1$). Experiment 2A found no evidence of differential responding in a discrete cue biconditional discrimination. This finding is consistent with the results of Experiment 5 in Fetsko et al. (2005).

4. Experiment 2B

Using the same method for sequencing trials as Experiment 2A, Experiment 2B examined the effect of simply doubling the number of reinforced and nonreinforced trials per session to match the number presented each day in Experiment 1. Additionally, with fewer subjects than Experiment 2A, Experiment 2B only partially counterbalanced the stimulus combinations but in all other respects was the same as Experiment 2A. The question of interest was whether acquisition of the discrete cue biconditional discrimination would emerge when the number of trials per session was the same as that of Experiment 1.

4.1. Methods

4.1.1. Subjects—Eight experimentally naïve male C57BL/6J mice, approximately 50 days old at the start of the experiment, served as subjects. The conditions for housing and maintenance were the same as those in Experiment 1.

4.1.2. Apparatus—The apparatus was the same as that used in Experiment 2A.

4.1.3. Procedure

4.1.3.1. Magazine training: This was the same as that given in Experiment 2A.

4.1.3.2. Biconditional discrimination training: There were 60 days of biconditional discrimination training. Training was identical to Experiment 2A with two exceptions. First, the number of trials in each session was increased from 16 to 32, thus matching Experiment 1. Second, all subjects received reinforced presentations of T and nonreinforced presentations of N during L1 (L1-T+, L1-N-) and reinforced presentations of N and nonreinforced presentations of T during L2 (L2-N+, L2-T-). Acquisition was measured as in Experiment 2A by comparing responding on reinforced and nonreinforced trials.

4.2. Results and Discussion

4.2.1. Biconditional discrimination training—Figure 4 shows mean rates of responding during the auditory cues on reinforced and nonreinforced trials in blocks of four daily sessions. As in Experiment 2A, response rates increased gradually and nondifferentially on both reinforced and nonreinforced trials. At the end of 60 days of training with double the number of trials per day used in Experiment 2A and the same number in Experiment 1, there was still no evidence of differential responding between reinforced and nonreinforced trials. A repeated measures ANOVA on the final block of four sessions found no significant main effect of Reinforcement ($F < 1$). These results underscore the difficulty that mice have in solving a Pavlovian biconditional discrimination with temporally asynchronous discrete cues. They also suggest that the relative ease with which the contextual biconditional discrimination was acquired in Experiment 1 is unlikely to be the result of the trial sequence per se or the number of trials per session.

5. Experiment 3

A technique that has proven extremely effective in facilitating the acquisition of biconditional discriminations with instrumental responses is the use of different rewards for correct responses (Brodigan & Peterson, 1976; Carlson & Wielkiewicz, 1976; Colwill, 1994; Kruse & Overmier, 1982; Trapold, 1970). First reported by Trapold (1970), the differential outcomes effect denotes superior acquisition of a discrimination in which one response earns one rewarding outcome in the presence of one stimulus and a different response earns a different rewarding outcome in the presence of another stimulus, relative to control groups that receive either the same outcome or mixed (nondifferential) outcomes for correct responses for all correct responses regardless of stimulus identity. Recently, Delamater et al. (2010) demonstrated a differential outcomes effect in a Pavlovian biconditional discrimination using rats. Like our discrete cue version, they used temporally asynchronous compounds but ones in which 10-sec auditory targets were embedded in 2-min presentations of flashing or steady lights.

Experiment 3 examined whether a differential outcomes manipulation might facilitate acquisition of the discrete cue Pavlovian biconditional discrimination used in Experiments 2A and 2B and by Fetsko et al. (2005) in mice. For all mice, the presentation of a white noise (N) was paired with a food US in the presence of an overhead light (L1) but not in the presence of a panel light (L2). The presentation of a tone (T) was paired with a food US in the presence of L2, but not in the presence of L1. For 16 mice assigned to Group D, differential outcomes were arranged for the reinforced compounds so that Polycose was paired with one reinforced compound and food pellets were paired with the other reinforced compound. For eight mice assigned to Group N, each reinforced compound was followed by food pellets on half of all trials and followed by Polycose on the remaining half of all trials. Acquisition was assessed by comparing responding on reinforced and nonreinforced trials.

5.1. Methods

5.1.1. Subjects—The subjects were 24 experimentally naïve male C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) approximately 50 days old at the start of the experiment. Housing conditions and feeding regimens were identical to those used in Experiment 1. Eight mice were randomly assigned to Group N and 16 mice to Group D.

5.1.2. Apparatus—The apparatus was that of Experiment 2A with one addition: A small recess in the magazine allowed access to a 0.1 ml dipper cup containing a solution of 15% Polycose (Abbott Laboratories, Columbus, OH) dissolved in spring water.

5.1.3. Procedure

5.1.3.1. Magazine training: Subjects were given two sessions of magazine training in which 15 food pellets were delivered on a VT 60-s schedule and two sessions of magazine training in which Polycose was presented 15 times on a VT 60-s schedule. During the first session with Polycose, the dipper cup was available for 30 seconds for the first 5 trials, 15 seconds for the next 5 trials, and 10 seconds for the last 5 trials. During the second session with Polycose, the dipper cup was available for 10 seconds on all 15 trials. The order of magazine training sessions was pellets, Polycose, Polycose, and pellets.

5.1.3.2. Biconditional discrimination training: There were 60 sessions of biconditional discrimination training. Each session contained 16 trials with a mean ITI of 150 sec. On each trial, a 15-sec light (L1 or L2) was presented accompanied in the last 5 sec by an auditory stimulus (N or T). Two stimulus combinations were reinforced (L1-N+ and L2-T+) and two were not reinforced (L1-T- and L2-N-). Each of these four trial types occurred equally often and in a random order within the first 8 trials of each session; the order of trials in the second half of a session was a mirror image of the order in the first half. For all animals, L1 was the houselight and L2 was the panel light.

The identity of the food rewards paired with the reinforced stimulus compounds was varied across animals. For eight animals in Group D, a single pellet was presented on L1-N+ trials and Polycose was presented for 10 sec on L2-T+ trials; for the other eight animals in Group D, these pairings were switched so that a single pellet was presented on L2-T+ trials and Polycose was presented for 10 sec on L1-N+ trials. For the eight animals in Group N, a single pellet was delivered on a randomly selected half of L1-N+ trials and a randomly selected half of L2-T+ trials. On the other half of each trial type, Polycose was presented for 10 sec. Thus, for Group D the stimulus compounds signaled differential outcomes and for Group N the stimulus compounds signaled nondifferential outcomes.

5.2. Results and Discussion

5.2.1. Biconditional discrimination training—Figure 5 shows mean responses per minute in blocks of four days to the auditory stimuli accompanied by L1 or L2 on reinforced and nonreinforced trials for Groups D and N. Both groups displayed a steep rise in the rate of responding at the start of training on both trial types. However, whereas Group N continued to respond nondifferentially throughout training, differential responding emerged in Group D. By the end of training, Group D was responding at a higher rate on reinforced trials than on nonreinforced trials. Data were collapsed across outcome identity because this factor was not statistically significant. During the last block of training, the mean rates of responding for Group D were 27.1 (SEM = 3.8) responses per minute during reinforced trials and 16.5 (SEM = 3.2) responses per minute during nonreinforced trials. For Group N, the mean rates of responding were 22.0 (SEM = 8.0) responses per minute during reinforced trials and 21.3 (SEM = 7.3) responses per minute during nonreinforced trials. Analysis of the last block of training with a mixed models ANOVA revealed a significant Reinforcement ×

Group interaction, $F(1,22) = 6.04$, $p < .05$. Follow up analyses revealed that Group D responded significantly more to the reinforced compounds than to the nonreinforced compounds, $F(1,15) = 14.97$, $p < .01$, but Group N showed no difference, $F < 1$. There was also no evidence that response rates on reinforced and nonreinforced trials differed significantly as a function of the identity of the visual stimulus (L1 or L2) for Group D in the last block of training. A repeated measures ANOVA found a significant main effect of Reinforcement, $F(1,15) = 14.97$, $p < .01$ but no main effect of Light ($F < 1$) and no significant Reinforcement $\times$ Light interaction, $F(1,15) = 2.98$, $p > .10$. This pattern of results showing no effect of stimulus identity is similar to that obtained in Experiment 1. In summary, the results of Experiment 3 indicate that differential outcomes facilitate acquisition of a Pavlovian biconditional discrimination with temporally asynchronous compounds in mice.

6. General Discussion

Four experiments examined Pavlovian biconditional discrimination learning in mice. Experiments 1 and 3 offer compelling evidence that mice can solve contextual and discrete cue versions of a Pavlovian biconditional discrimination. Experiment 1 found differential responding in a contextual biconditional discrimination using the same US (food pellets) on all reinforced trials. Experiment 3 found differential responding in a discrete cue biconditional discrimination with differential outcomes (food pellets and Polycose) for the two reinforced compounds. These findings complement previous reports of successful acquisition of instrumental biconditional discriminations in other mouse strains (Barnes et al., 2004; Dickson et al., 2010; Dreumont-Boudreau et al., 2006).

However, Experiment 3 also found no evidence of differential responding on reinforced and nonreinforced trials in the control group receiving mixed (nondifferential) outcomes on reinforced trials. Furthermore, using the same US (food pellets) on all reinforced trials, Experiments 2A and 2B yielded no evidence of differential responding on a discrete cue biconditional discrimination in which the scheduling of different trial types modeled that used in conventional contextual biconditional discriminations. These findings confirm the results of Fetsko et al. (2005, Experiment 5) which also failed to demonstrate differential responding on a discrete cue biconditional discrimination using the same outcome for both reinforced compounds.

Fetsko et al. (2005) interpreted their failure to find differential responding on reinforced and nonreinforced trials in a discrete cue biconditional discrimination in terms of Rescorla's (1985) US threshold modulation account of facilitation. This model was developed to explain the augmentation of responding to a target cue by a facilitator, a stimulus which signals when the target will be paired with an US, as well as by facilitators trained with other targets and that US. According to the US threshold modulation account, facilitation training establishes the facilitator with the ability to lower the threshold for activation of the US representation by any stimulus associated with that US (Rescorla, 1985). Fetsko et al. (2005) attributed the lack of differential responding on their biconditional task to a transfer effect because the two lights (L1 and L2) promoted responding to each other's reinforced targets as a result of their shared association with the same US.

In addition, the US threshold modulation account predicts that using different outcomes for the two reinforced compounds in a biconditional task should undermine the basis for transfer effects. The results of Group D in Experiment 3 confirm this prediction. When food pellets followed one reinforced compound and a Polycose liquid followed the other reinforced compound, differential responding emerged between reinforced and nonreinforced trials in the Pavlovian biconditional discrimination. Moreover, as to be expected, when these outcomes were mixed as in Group N, differential responding did not emerge just as it had

failed to emerge in Experiments 2A and 2B with a single outcome for both reinforced compounds.

If failures to obtain evidence of Pavlovian biconditional discrimination learning with a single outcome are the result of transfer, how then do we accommodate reports of successful acquisition of Pavlovian biconditional discriminations using a single outcome within Rescorla's (1985) US threshold account? A general solution to this problem might assert that an US is represented differently in different contexts and that these successful demonstrations reflect a differential outcomes effect equivalent to that observed in Group D of Experiment 3. For instance, information about the physical context or location in which a food outcome is presented could be incorporated into the representation of that outcome making it unique. Consistent with this idea are studies showing that taste aversion learning and latent inhibition effects in taste aversion learning can be context specific (Archer, Sjöden, & Carter, 1979; Archer et al., 1985; Archer, Sjöden, Nilsson, & Carter, 1979; Hall & Channell, 1986; Ward-Robinson et al., 1998) and that a differential outcomes effect is not dependent on USs that differ in hedonic value (Kelly & Grant, 2001; Miller et al., 2009). Alternatively, different contexts might directly impact delivery or retrieval of the food US. For example, in Experiment 1, the Plexiglas floor inserts used to distinguish Context A from Context B decreased the space between the floor and the top of the food magazine by approximately 25 percent. An unintended consequence was that a mouse had to reach down to retrieve the food pellet in Context A but only reach forward to retrieve the pellets in Context B. The notion that variations in the mode of outcome delivery can affect the representation of that outcome has been used to explain residual responding following outcome devaluation (Colwill, 1994; Colwill and Rescorla, 1985, 1986). Moreover, Williams et al. (1990) obtained a differential outcomes effect in pigeons simply by varying the spatial location (high or low) of the food US. However, this approach may not be so plausibly applied to differential responding in studies using a single outcome with either sequential nonoverlapping biconditional compounds (Honey and Watt, 1998, 1999) or temporally abbreviated pseudocontexts (Wilson and Pearce, 1989).

In contrast to the US threshold modulation account, the original modulatory approach developed by Holland (1983, 1985) to explain occasion-setting phenomena like facilitation requires no special assumption to be made about contextual effects on outcome encoding to accommodate Pavlovian contextual biconditional discriminations with a single outcome. According to this view, the first or longer element of a compound stimulus (the occasion-setter) is thought to modulate the relationship between the second or embedded element (the target) and the US (Holland 1983, 1985). Within this framework, the critical feature determining successful acquisition of Pavlovian biconditional discriminations is the degree to which the reinforced compounds can be discriminated from the nonreinforced compounds. For example, the basis for the difference between the contextual biconditional discrimination in Experiment 1 and the discrete cue biconditional discrimination used in Experiments 2A and 2B would be attributed to variations in the discriminability of the occasion-setters, there being greater similarity of the two lights than of the two floors. In Experiment 3, the difference between Group D and Group N would be attributed to the relative similarity of the target-US associations, there being greater generalization between the targets associated with the same US than the targets associated with different USs.

We have outlined how two different modulatory frameworks can capture the present results and those of Fetsko et al. (2005). Our focus on modulatory rather than configural mechanisms seems appropriate because we deliberately tried to encourage modulatory strategies in our experiments by using stimuli of different durations (Holland, 1986, 1992; Rescorla, 1985; Ross & Holland, 1981) and from different modalities, and so discourage configural conditioning (Melchers et al., 2008). Recent work in our laboratory has also

found that removing the temporal asynchrony of the compound elements enables mice to respond differentially in a Pavlovian biconditional discrimination when the same outcome follows the two reinforced compounds. Colwill (2011) reported successful acquisition of a discrete cue biconditional discrimination using a pellet outcome and visual-auditory compounds that were 10 sec in duration. Further research is needed to determine precisely how the temporal arrangements of the compound elements influence the strategy used to solve a Pavlovian biconditional discrimination and the discriminability of those stimulus compounds.

In general, Pavlovian biconditional discriminations are notoriously difficult for animals to learn. Our experiments suggest that this task is made more tractable for mice by using differential outcomes for the two reinforced compounds (Experiment 3) when temporally asynchronous cues are used. This manipulation should also facilitate acquisition of a biconditional task with temporally contiguous cues because the elements in the nonreinforced compounds are not associatively equivalent to those in the reinforced compounds thereby eliminating the need to depend on a unique cue or a configural solution. Our data also suggest that using contextual stimuli (Experiment 1) can make biconditional discriminations easier. Further work is needed to determine if this result reflects a covert differential outcomes effect, the use of more discriminable stimuli, an effect of blocked trials, or the operation of a different learning strategy. For example, consider the striking similarity between a contextual biconditional discrimination and the ABA renewal procedure developed by Bouton and his colleagues (Bouton & Bolles, 1979; Bouton & King, 1983; Bouton & Peck, 1989). In their procedure, a Pavlovian CS is paired with an US in Context A and then extinguished in Context B. When the CS is returned to Context A, responding is renewed, indicating that extinction is context specific. They attribute this result to the development of an inhibitory association between Context B and the CS-US association. If contextual biconditional discriminations are also solved in this way, we should expect to see little benefit of using explicitly different outcomes on acquisition.

In this paper, we report two novel findings about biconditional discrimination learning in mice. Experiment 1 provides the first demonstration of successful acquisition of a contextual Pavlovian biconditional discrimination. Experiment 3 provides the first demonstration that acquisition of a Pavlovian biconditional discrimination with temporally asynchronous discrete elements can be facilitated by using different outcomes for the two reinforced compounds. Continued assessment of the circumstances under which discrimination learning is affected by cue onset asynchrony and outcome identity will be important for a full appreciation of the learning strategies animals use to make sense of the events that occur in their environment.

Highlights

> C57BL/6J mice exhibit Pavlovian biconditional discrimination learning > The use of contextual cues and differential outcomes facilitate this type of discrimination learning > Mice did not solve discrete cue biconditional discriminations with nondifferential outcomes > Various modulatory analyses can account for these discrepancies in learning

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Figure 1.

Experiment 1. Mean responses per minute throughout contextual biconditional discrimination training to all reinforced (+) and nonreinforced (-) compounds.

Figure 2.

Experiment 1. Panel A: Assessment of control by designated contextual cues. Mean responses per minute to the reinforced (+) and nonreinforced (–) compounds. Congruent refers to the condition in which the Plexiglas floor inserts were in boxes 1–4 and the grid floors were in boxes 5–8, as they were throughout training; Incongruent refers to the condition in which the grid floors were in boxes 1–4 and the Plexiglas floor inserts were in boxes 5–8. Panel B: Assessment in extinction. Mean responses per minute to the reinforced (+) and nonreinforced (–) compounds in the last session of retraining (bars on left) and in the extinction test (bars on right).

Figure 3. Experiment 2A. Mean responses per minute throughout biconditional discrimination training with temporally packaged discrete cues to all reinforced (+) and nonreinforced (-) compounds.

Figure 4.

Experiment 2B. Mean responses per minute throughout biconditional discrimination training with temporally packaged discrete cues to all reinforced (+) and nonreinforced (-) compounds.

Figure 5.

Experiment 3. Mean responses per minute throughout biconditional discrimination training to all reinforced (+) and nonreinforced (–) compounds. Data are presented separately for Group D which received differential outcomes and Group N which received nondifferential outcomes.

Basic Design of Experiment 1

Training		Assessment 1		Assessment 2	
Boxes	Boxes	Boxes	Boxes	Boxes	Boxes
1-4	5-8	1-4	5-8	1-4	5-8
A: N+, T-	B: T+, N-	A: N+, T-	B: T+, N- (Congruent)	A: N-, T-	B: T-, N-
		B: T+, N-	A: N+, T- (Incongruent)		
A: T+, N-	B: N+, T-	A: T+, N-	B: N+, T- (Congruent)	A: T-, N-	B: N-, T-
		B: N+, T-	A: T+, N- (Incongruent)		

Note: A and B are contexts; N and T are 10 sec presentations of a noise or tone, respectively. + indicates reinforced; - indicates nonreinforced. Specific combinations were counterbalanced across animals; The top rows show the contingencies for 8 mice and the bottom rows show the contingencies for 8 other mice.