Second-Order Conditioning of the Rabbit's Nictitating Membrane Response

Interstimulus Interval and Frequency of CS-CS Pairings

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Abstract—Second-order conditioning of the rabbit's nictitating membrane response (NMR) was investigated when second-order trials (CS1-CS2) were intermixed with first-order trials (CS2-US) from the outset of training. Experiment 1 showed that CR acquisition to CS1 was inversely related to the CS1-CS2 interval but nevertheless extended to an interval of 8,400 ms. Experiment 2 revealed that CR acquisition of CS1 was an inverted-U function of the number of CS1-CS2 trials relative to a fixed number of CS2-US trials. Experiment 3 directly contrasted second-order conditioning with a reinforced serial compound procedure (CS1-CS2-US) and a mixed procedure in which second-order trials were intermixed with the reinforced serial compound. Second-order conditioning was about half the strength of either the reinforced serial compound or the mixed procedure, which were similar. The present results are discussed with respect to the relative strength of excitatory and inhibitory processes in second-order conditioning.

THE PURPOSE OF THE PRESENT EXPERIMENTS was to delineate the strength of second-order conditioning with intermixed CS1-CS2 and CS-US trials of the rabbit's nictitating membrane response (NMR) as a function of: (a) the CS1-CS2 interval (Experiment 1); (b) the number of CS1-CS2 pairings (Experiment 2); and (c) interspersed nonreinforced CS1-CS2 pairings among reinforced CS1-CS2-US serial compound trials (Experiment 3).

The facilitated acquisition of CRs to a trace-CS1 by the insertion of a second stimulus, CS2, just prior to US onset in the serial compound CS1-CS2-US, has been observed in autoshaping (Kaplan & Hearst, 1982; Rescorla, 1982), conditioned suppression (Kamin, 1965; Pearce, Nicholas, & Dickinson, 1981), and NMR conditioning (Kehoe, Gibbs, Garcia, & Gormezano, 1979; Kehoe, Marshall-Goodell, & Gormezano, 1987). Although serial compound conditioning to CS1-CS2-US, would appear to involve only a slight increase in complexity over trace-CS1-US conditioning, there are five broad mechanisms by which

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CS2 could operate to augment CS1 responding (see Gormezano & Kehoe, 1989): direct conditioning to CS1 and CS2; serial mediation governed by the mere presence of CS2; and stimulus generalization, general transfer, and associative transfer from CS2 to CS1. Our experimental efforts to assess the potential contribution of these mechanisms to augmented CS1 responding in serial compounds have focused on three interstimulus interval relations: CS1-CS2, CS1-US, and CS2-US (Gormezano & Kehoe, 1984; Kehoe, Cool, & Gormezano, in press; Kehoe, Feyer, & Moses, 1981). Our studies have revealed that (a) the processes of direct conditioning, stimulus generalization, and general transfer, as estimated jointly by the use of intermixed CS1-US and CS2-US trials, can account for a portion of CR acquisition to CS1 in a serial compound; and (b) associative transfer, as estimated by the use of intermixed CS1-CS2 and CS2-US trials, can produce acquisition to CS1, but not to the same extent as comparable serial compounds (Kehoe et al., 1979; Kehoe & Morrow, 1984). However, the second-order conditioning procedure of intermixed CS1-CS2 and CS2-US pairings, provides the necessary conditions for CS1 to acquire inhibitory properties (Herendeen & Anderson, 1968; Pavlov, 1927) and, thereby, may underestimate the strength of associative transfer to CS2. Nevertheless, the second-order conditioning paradigm remains the key tool for the study of associative transfer in serial compounds. Accordingly, the focus of the present experiments was to detail key determinants of second-order conditioning.

Experiment 1

The present experiment sought to determine the effect on CS1 responding of CS1-CS2 pairings at intervals of 400, 1,400, 2,40, 4,400, and 8,400 ms and intermixed CS2-US pairings at a fixed interval of 400 ms. To control for cross-modal generalization effects from CS2 to CS1 and any nonassociative contributions arising from the US, the study contained a group (Group UP), which received unpaired presentations of CS1 and CS2 interspersed among CS2-US trials.

Method

Subjects

The subjects were 72 naive male and female albino rabbits (*Oryctolagus cuniculus*), each 80 to 100 days old and weighing about 2.0 kg on arrival. The animals had free access to food and water in their home cages.

Apparatus

The apparatus and recording procedure for the NM response were those described by Coleman and Gormezano (1971), who detail modifications of the apparatus described by Gormezano (1966). In brief, each subject was restrained in a Plexiglas box and trained individually in one of 12, sound-attenuating, ventilated conditioning chambers fabricated from legal-sized, fireproof filing cabinets. A speaker was mounted vertically 15 cm anterior to and 10 cm above the subject's head.

CS1 was a 400-ms, 1000-Hz, 82-dB (SPL) tone superimposed on a 68-dB background provided by chamber fans, and CS2 was a 400-ms, 10-Hz flashing of two 6-W, 24-V houselights, which resulted in a change in illumination from 32 lx to 8 lx at the animal's eye level. The US was a 100-ms, 3-mA, 60-Hz electrotactile stimulus delivered through two 9-mm stainless-steel wound clips, positioned 15 mm apart and 10 mm posterior to the dorsal canthus of the rabbit's right eye. To transduce NM movements (Gormezano & Gibbs, 1988), a small hook was attached to a nylon loop sutured in the NM of the rabbit's right eye. The hook was connected to one end of an L-shaped hypodermic tubing lever. Inside the transducer, movement of the lever rotated a disk of polarized filter, which was interposed between a light-emitting diode and a photoresistor covered by a fixed polarized filter. Thus, rotation of the disk produced changes in the intensity of the light reaching the photoresistor through the fixed filter. The signal from the photoresistor was amplified and topographical characteristics recorded.

Procedure

All rabbits received 1 day of preparation, 1 day of recovery, and 1 day of adaptation. On the preparation day, hair surrounding the rabbit's right eye was removed, a small loop of surgical nylon (Ethicon 4-0) was sutured into the NM, the surrounding hair was removed, and the US electrodes were applied. On the adaptation day, the animals were placed in the conditioning apparatus for 70 minutes, but neither a CS nor US was presented. The base rate of NM movements was obtained by observations made at times corresponding to the CS-US interval used during subsequent training. Following adaptation, the animals were assigned randomly to one of six groups ($n = 12$) for 16 days of training. Five second-order conditioning groups received 30 CS1-CS2 pairings at interstimulus intervals (ISIs) of 400, 1,400, 2,400, 4,400 and 8,400 ms, respectively, and all groups received 30 CS2-US pairings at an ISI of 400 ms. These second-order conditioning groups were designated by their respective CS1-CS2 trace intervals between the offset of CS1 and onset of CS2 of 0, 1, 2, 4, and 8s. Thus, these groups were designated as Groups P-0, P-1, P-2, P-4, and P-8. In all five groups, the intertrial intervals (ITIs) were 70, 90, and 110s (mean = 90s). The sixth group, Group UP, received 30 paired CS2-US and 30 unpaired CS1 and CS2 trials presented randomly at intertrial intervals of 40, 45, or 50s (mean = 45s). All groups received two daily nonreinforced test trials of CS1 and CS2 on every 14th trial.

Response Definition

On paired trials, a CR was defined as any NMR extension exceeding 0.5 mm within the 400-ms duration of CS1 or CS2. On CS1 and CS2 test trials, the observation interval was extended to 8,400 ms to allow equal opportunity to observe CRs across all groups.

Results

Figure 1 shows the mean CR percentages of CS1 (left-hand panel) and CS2 (right-hand panel) as a function of 4-day blocks. Examination of the left-hand panel reveals that all second-order conditioning groups showed evidence of CR acquisition to CS1 as an inverse

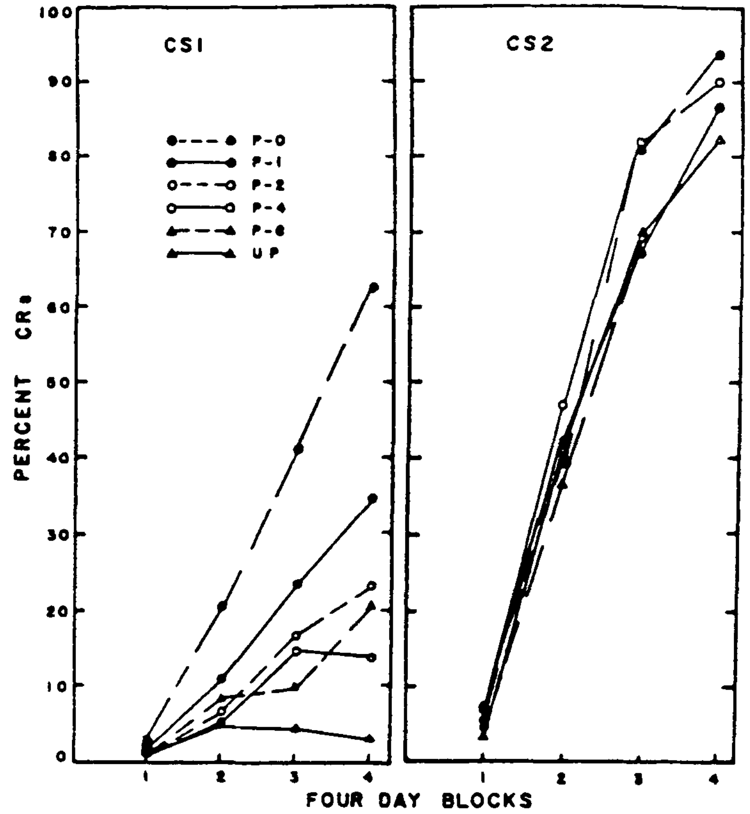

FIG 1. The mean percentage of NM CRs to CS1 (left-hand panel) and CS2 (right-hand panel) on test trials, over four-day blocks for all six groups in Experiment 1.

function of the CS1-CS2 interval. Specifically, responding to CS1 in Groups P-0, P-1, P-2, P-4, and P-8 rose steadily to terminal levels of 64%, 35%, 24%, 16%, and 20% CRs, respectively. In contrast, Group UP produced no evidence of CR acquisition to CS1; its response level hovered around a mean of 3%. The functional relationship between CS1-CS2 interval and CS1 responding across the 16 days of acquisition training is present in Figure 2. The figure reveals that CS1 responding was a negatively decelerating function of the CS1-CS2 trace interval. The function is characterized by a 16% reduction in CRs to CS1 at CS1-CS2 trace intervals of 0 to 1,000 ms, a 6% reduction at intervals of 1,000 to 2,000 ms, and a stabilization of responding at about 8 to 10% CRs at the 1,000 to 2,000 ms, which is substantially higher than the 3% level of Group UP. Finally, an examination of the right-hand panel of Figure 1 reveals that all groups showed steady CR acquisition to CS2, reaching essentially comparable mean levels of around 90% CRs by the end of training.

An analysis of variance of responding to CS1 confirmed the descriptive aspects of the data displayed in Figures 1 and 2. Specifically, the analysis yielded a significant effect of Groups, $F(5, 66) = 9.00, p < .01$, and a Groups x Blocks interaction $F(15, 198), p < .01$. To identify which groups revealed the acquisition of CRs to CS1, a test for the simple effect of blocks was applied separately to each group. These tests yielded a significant effect ($p < .01$) of Blocks in Groups P-0, P-1, P-2, P-4, P-8, but not in Group UP, Fs $(3, 198) = 81.43$, 24.87,

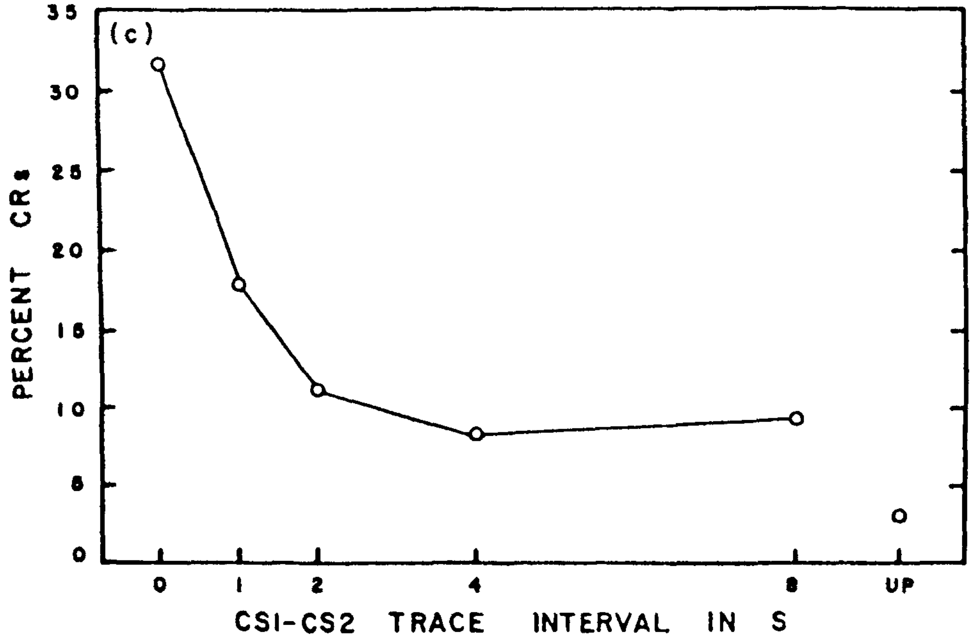

FIG 2. The mean percentage of NM CRs to CS1 on text trials as a function of the CS1-CS2 interval. Performance under the unpaired (UP) condition is treated as the extreme point on the CS1-CS2.

12.73, 5.50, 7.10, and < 1, respectively. Further Tukey *hsd* comparisons, critical difference [(6,150) = 16%, a = .05)] applied to the final block confirmed that responding to CS1 in all five conditioning groups, except Group P-4 was greater than in Group UP. Moreover, Group P-0 groups, except Group P-4 was greater than in Group UP. Moreover, Group P-0 revealed a higher frequency of CRs to CS1 than all the other groups. On the other hand, any noticeable differences in the frequency of CRs to CS2, displayed in the right-hand panel of Figure 1, failed to approach statistical significance.

Discussion

The principal findings of the present investigation were: (a) second-order conditioning to CS1 was acquired at the CS1-CS2 trace intervals of Groups P-0, P-1, P-2, P-4, and P-8; (b) second-order conditioning to CS1 was a negatively decelerated function of CS1-CS2 trace interval; and (c) first-order conditioning of CRs to CS2 was unaffected by the CS1-CS2 trace interval.

Frolov's second-order conditioning research in Pavlov's (1927) laboratory, which was neither systematic nor compelling, led Pavlov to conclude that second-order conditioning was not a robust phenomenon. Nevertheless, the employment of higher-order conditioning as a postulated process to account for goal-directed instrumental behavior (e.g., Amsel, 1958; Gormezano, 1980; Hull, 1930, 1931, 1934) has nurtured a continued theoretical interest in second-order conditioning but only in recent years has it begun to receive empirical support. The contemporary studies of second-order conditioning involved classical-instrumental (CS-IR) (e.g., Herendeen & Anderson, 1968; Rizley & Rescorla, 1972) and autoshaping (e.g., Rashotte, Griffen, & Sisk, 1977) paradigms; whereas, subsequent second-order conditioning with traditional classical conditioning (CS-CR) paradigms involving the direct measurement of CRs has only been reported for the rabbit NMR preparation (Kehoe, Feyer, & Moses, 1981; Kehoe & Morrow, 1981). Nevertheless, there still remains a paucity of data for adequately delineating the empirical laws of second-order conditioning. In this regards, the present results have clearly specified a robust second-order contiguity gradient with implications in regard to first-order laws of conditioning. In particular, the CS-US contiguity gradient for conditioning the rabbit NMR to a single-CS has been demonstrated repeatedly to have an outer boundary of no more than 4,000 ms under present and similar conditions (cf. Gormezano, 1972); whereas, the presently observed CRs to CS1 at CS1-CS2 trace intervals up to 8,000 ms suggests that first- and second-order contiguity gradients may be fundamentally different. Furthermore, the finding that CRs to CS1 were not significantly different from one another at the 2,000, 4,000, and 8,000 ms CS1-CS2 intervals but were significantly superior to controls, suggests that the outer boundary of the second-order contiguity gradient had not yet been reached. In any event, the extended nature of the second-order contiguity gradient parallels that obtained for CS1 responding under serial compound conditioning (CS1-CS2-US), but the absolute level of second-order conditioning to CS1 appears to be substantially (50%) less than in the serial compounds (Kehoe et al, 1979; Kehoe et al., 1981; Kehoe & Morrow, 1984). Accordingly, as an estimate of associative responding to CS1 in serial compounds, the present findings suggest that second-order conditioning provides only a partial account.

Experiment 2

The present experiment examined CR acquisition to CS1 when the number of CS1-CS2 presentations per session was manipulated. At an empirical level, the aim was to identify the optimal proportion of CS1-CS2 trials to CS2-US trials for promoting second-order conditioning. For theoretical purposes, the aim was to discover whether there is a trade-off between the excitatory and the inhibitory effects of the second-order procedure on CS1 responding.

There are at least two reasons that point toward a trade-off between the two opposing processes. First, attempts to estimate the parameters for reinforcement and nonreinforcement for various models of conditioning have suggested that the reinforcement parameter is higher than the nonreinforcement parameter in the rabbit NMR preparation (e.g., Bellingham, Gilette-Bellington, & Kehoe, 1985; Kehoe, 1988). Second, in studies using intermixed CS1-CS2 and CS2-US trials, sustained training has revealed that responding to CS1 follows a curvilinear course (i.e., an initial rise followed by an eventual decline in CR frequency) (Herendeen & Anderson, 1968; Kehoe et al., 1981). The rise is thought to represent excitatory conditioning arising from associative transfer, and the decline is thought to reflect the more gradual encroachment of the inhibitory process.

Method

Subjects

The subjects were 96 naive male and female albino rabbits, each 80 to 100 days old and weighing about 2.0 kg on arrival.

Apparatus and Procedure

Unless otherwise specified, the apparatus and procedure were as described for Experiment 1. Following surgical preparation, the rabbits were randomly assigned to one of eight groups ($n = 12$) for 16 days of training. All groups received 30 CS2-US trials within each 90-minute daily session. The CSB-US interval was 400 ms. The distribution of CS2-US trials within each session was identical for all groups. The interval between successive CS2-US trials were 180 s (range: 80–270 s).

Four groups were designated as Groups P5, P15, P25 and P50. Within each session they received 5, 15, 25, and 50 second-order CS1-CS2 pairings, respectively. These CS1-CS2 trials were randomly interspersed among the CS2-US trials with, however, no more than two CS1-CS2 presentations falling between successive CS2-US trials. The CS1-CS2 interval was 1,400 ms in all four groups. The other groups were designated U5, U15, U25, and U50. Within each session, they received 5, 15, 25, and 50 explicitly unpaired presentations of CS1 and CS2 respectively. In the unpaired groups, the placement of the CS1 presentations matched that of the corresponding paired group with the presentations of CS2 occurring prior to CS1 and following CS1 on an equal number of occasions. The mean interval between unpaired CS1 and CS2 presentations was 45 s (range: 40–50 s).

Results

The upper and lower left-hand panels and the right-hand panel of Figure 3 show the mean percentages of CRs in 2-day blocks to CS1 and CS2, respectively, for the paired and unpaired groups. An examination of responding to CS1 reveals two key features. First, evidence of successful second-order conditioning was revealed by the higher levels of CS1 responding in the paired rather than in the unpaired groups, $F(1, 88) = 11.97, p < .01$. Second, the number of paired or unpaired CS1 and CS2 presentations produced inverted-U shaped CS1 response functions. An analysis of variance revealed a significant effect of number, $F(3, 88) = 5.08, p < .01$ and a subsequent trend test yielded significant linear and quadratic trends in responding to CS1 across the number of CS1-CS2 trials per session, $F\text{s}(1, 88) = 4.94, p < .05, 10.23, p < .01$. The pattern of differences in responding to CS1 were consistent throughout training. For example, the terminal levels for Groups P5, P15, P25, and P50 were 16%, 30%, 31%, and 5% CRs, respectively. In a parallel fashion, the asymptotic levels for Groups U5, U15, U25, and U50 were 6%, 9%, 20%, and 2% CRs, respectively. With respect to CS2 responding, all groups except Group U50, showed similar high levels of responding. Notwithstanding Group U50's substantially lower performance, statistical analysis revealed a significant main effect of Number, $F(3, 88) = 3.28, p < .05$, on CS2 responding and a significant interaction of Number X Blocks $F(21, 88) = 1.68, p < .05$ that primarily reflected the decline in CS2 responding of Group P50 toward the terminal stages of training.

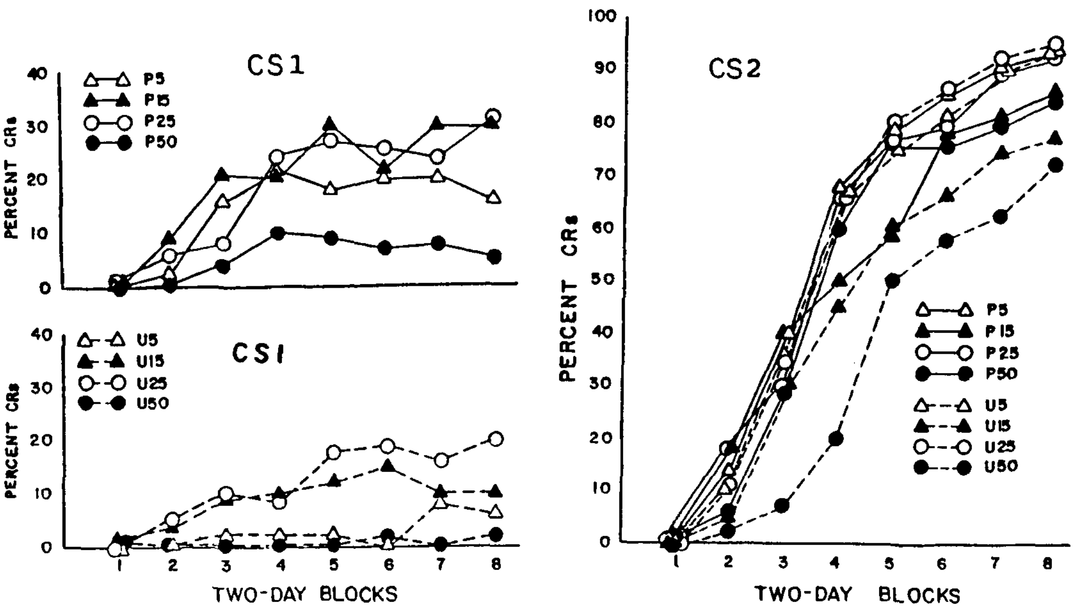

FIG 3. The mean percentage of NM CRs to CS1 (upper and lower left-hand panels) and CS2 (right-hand panel) for paired and unpaired groups as a function of number of CS1-CS2 trials in two-day blocks in Experiment 2.

Discussion

The major findings of the present experiment were that CS1 responding was a concave-downward function of the number of trials with higher levels of responding under paired than unpaired conditions. The finding that responding to CS1 was greater under the paired CS1-CS2 than unpaired CS1/CS2 condition substantiates the occurrence of second-order conditioning across a relatively wide range of CS1-CS2 pairings with a training regime involving intermixed presentations of first- and second-order conditioning trials. In the axiomatic application of higher-order conditioning to CR-mediational accounts of instrumental conditioning, a second-order conditioning must be demonstrated to persist for extended numbers of trials. Yet, the large majority of second-order conditioning studies have employed relatively few CS1-CS2 trials and, thus, are only marginally relevant to CR-mediation accounts (e.g., Green & Schweitzer, 1976, 8 trials; Holland & Rescorla, 1975, 16 trials; Maisiak & Frey, 1977, 42 trials; Rashotte, Griffin & Sisk, 1977, 40 trials). In the only prior study involving manipulation of the number of CS1-CS2 trials (Herendeen & Anderson, 1968), inhibition appeared to accrue to CS1 with 200 CS1-CS2 trials, but a determination of CS1's inhibitory properties through summation and retardation testing (Rescorla, 1969) was not determined.

The present and previous experiment indicated that second-order CRs can persist for a substantial number of CS1-CS2 trials in spite of the discriminative nature of intermixing nonreinforced CS1-CS2 and reinforced CS2-US trials. The present experiment also demonstrated that increasing the number of CS1-CS2 trials did have a detrimental effect on CS1 responding with Group P50 demonstrating responding substantially lower than the other paired groups throughout the course of the experiment. The nonmonotonic CS1 response function cannot be attributed to differences in CS2 responding since all paired groups showed virtually the same levels of CS2 responding.

Although the nonmonotonic function seen in the paired groups is consistent with the hypothesis that second-order conditioning is counteracted by conditioned inhibition accruing to CS1 on the nonreinforced CS1-CS2 trials, the parallel function seen in the unpaired groups suggests that other processes are at work. Specifically, the unpaired groups, included to estimate the level of cross-modal generalization from the visual CS2 to the auditory CS1, would be expected to depend on the level of responding to CS2. Thus, the modest decline in CS2 responding across the CS1-CS2 trials could explain the decline in CS1 responding between the U25 and U50 groups. Yet, it remains unclear how a generalization hypothesis would explain the apparent increase in responding to CS1 across 5, 15, and 25 unpaired presentations per day when responding to CS2, being relatively constant, would lead to the expectation of uniform generalization to CS1. However, with respect to promoting second-order conditioning, the shape of the function does not matter a great deal, because no group showed particularly high levels of responding to CS1. In terms of identifying boundary conditions for responding to CS1, it would appear that 50 CS1-CS2 trials per session did have a consistent deleterious effect on responding to both CS1 and CS2 in both paired and unpaired groups, which may explain why it might have been difficult to see the presumed trade-off between the excitatory (CS2-US) and inhibitory (CS1-CS2) effects of training under the paired condition. Specifically, under conventional accounts of conditioning, both excitatory and inhibitory conditioning of CS1 would depend on the level of excitatory conditioning of CS2. Thus, early in training, when excitatory conditioning of CS2 was weak, there was little excitation to pass on to CS1 through associative transfer nor, by the same token, was the excitation on the nonreinforced CS1-CS2 trials strong enough to engage the inhibitory process. Furthermore, throughout training, the partial reinforcement of CS2 in the second-order procedure would tend to dampen excitatory conditioning of CS2, thus further hindering both the excitatory and inhibitory conditioning of CS1.

In summary, there appear to be two sources that depress CR acquisition to CS1 in second-order conditioning. First, partial reinforcement of CS2 tends to reduce the acquisition of first-order associative strength to CS2 and thereby hinders second-order conditioning. Second, the conditioned inhibition procedure embedded in the mixture of CS1-CS2 trials and CS2-US trials would serve to counteract excitatory conditioning of CS1.

Experiment 3

The present experiment undertook to compare the level of responding to CS2 under three key conditions: (a) second-order conditioning consisting of CS1-CS2 and CS2-US trials; (b) serial CS1-CS2-US compound conditioning; and (c) a hybrid procedure of second-order CS1-CS2 pairings interspersed among serial CS1-CS2-US compound conditioning trials. The hybrid procedure allowed the partial-reinforcement effects of CS1-CS2 trials to be separated from the potential occurrence of conditioned inhibition embedded in a second-order procedure. The hybrid procedure of second-order and serial compound trials allowed both CS1 and CS2 to receive 50% reinforcement; whereas, in the conventional second-order conditioning procedure, CS2 received 50% reinforcement, but CS1 received no reinforcement. The contrast between the first and second conditions provided the basis for a direct comparison between serial compound conditioning and second-order conditioning. Comparisons of these procedures within a single experiment has been limited to only one rabbit NMR conditioning investigation (Kehoe & Morrow, 1984). Furthermore, second-order conditioning has been demonstrated only once in a fully-controlled fashion in the rabbit NMR preparation (Kehoe et al., 1981) in contrast with the negative results obtained by Frey and his associates in the related rabbit eyeblink preparation (Maisiak & Frey, 1977; Popik, Stern, & Frey, 1979; Sears, Baker, & Frey, 1979). Accordingly, the present experiment includes a complete set of unpaired controls for second-order conditioning in order to unequivocally confirm the associative nature of second-order NMR conditioning.

Method

Subjects

The subjects were 72 naive male and female albino rabbits, each 80 to 100 days old and weighing about 2.0 kg on arrival.

Apparatus

Unless otherwise specified, the apparatus and recording procedure were as described for Experiment 1. The chambers were plywood boxes lined with white acoustical board covering fiberglass insulation. The interior dimensions were 63 cm x 32 cm x 50 cm. Stimulus panels were located 50 cm anterior to and 20 cm above the subject's head. CS1 was a 400-ms, 82-dB (SPL), 1,000-Hz tone superimposed on an ambient noise level of 70 dB provided by the chamber's ventilation fans and white noise. CS2 was a 400-ms, 10-Hz flashing of two 6-W, 24-V houselights, which produced a change in illumination at the subject's eye level from 6 lx to 1 lx.

Procedure

The animals were assigned randomly to six groups (n = 12). The three main groups were designated as SOC, SER, and MIX, and received training with the second-order, serial compound, and mixed procedures, respectively. During each training session, Group SOC received 30 CS1-CS2 trials intermixed with 30 CS2-US trials. Group SER received just 30 CS1-CS2-US trials in each session, and Group MIX received 30 CS1-CS2 trials intermixed with 30 CS1-CS2-US trials. In all three groups, the CS1-CS2 interval (onset to onset) was 1,400 ms, and the CS2-US interval (onset to onset) was 400 ms. Thus, on CS1-CS2-US trials, the CS1-US interval (onset to onset) was 1,800 ms. For these three groups, the ITIs were 60, 75, and 90 s (mean = 75 s).

The three remaining groups constituted a set of unpaired controls for second-order conditioning. Specifically, Group UP controlled for generalization from CS2 to CS1 by receiving 30 unpaired presentations each of CS1 and CS2 and 30 CS2-US pairings per session. Group PU controlled for any unknown consequences of CS1-CS2 pairings by presenting 30 CS1-CS2 pairings plus 30 unpaired presentations each of CS2 and the US per session. Finally, Group UU received during each session 30 CS1 presentations, 60 CS2 presentations, and 30 US presentations, all unpaired at ITIs of 30, 37.5, and 45 s (mean = 37.5 s); thus, this group represented a truly nonassociative control condition.

In all groups, except Group SER, the trials were presented in a randomized sequence subject to the constraint that no more than three of one type could occur in succession. In order to provide an equivalent basis for assessing responding to the components across groups, all groups received within each session, one presentation each of CS1 and CS2, which were located on an alternating basis one-third and two-thirds of the way through the trial sequence. With respect to CR measurement on nonreinforced test trials, the observation interval was 1,800 ms from onset for all stimuli.

Results

Figure 4 presents the mean percentage CRs on CS1 and CS2 test trials as a function of 4-day blocks. Examination of the left-hand panel reveals that responding to CS1 in the unpaired groups—Groups UP, PU, and UU—displayed uniformly low levels of responding (less than 10%) throughout training. In marked contrast, Groups SOC, MIX, and SER each demonstrated systematic increases in responding to CS1, reaching terminal levels of 37%, 64%, and 70% CRs, respectively. Clearly, Group SOC showed second-order conditioning when compared to the unpaired controls. On the other hand, Groups SOC's performance fell well below those of either Group MIX or Group SER, which themselves only showed modest differences. An overall analysis of variance on the data portrayed in the left-hand panel of Figure 4 yielded significant effects of Groups, $F(5, 66) = 16.53$, $p < .01$ and Groups X Blocks, $F(15, 198) = 4.61$, $p < .05$. Subsequent Tukey *hsd* comparisons for the last block of training confirmed that responding to CS1 in Groups MIX and SER was higher than in Group SOC which, in turn, was higher than in the unpaired groups, critical difference (6, 198, a = .05) = 27%. Any other apparent differences failed to attain significance.

Examination of the right-hand panel in Figure 4 reveals that CR acquisition to CS2 in

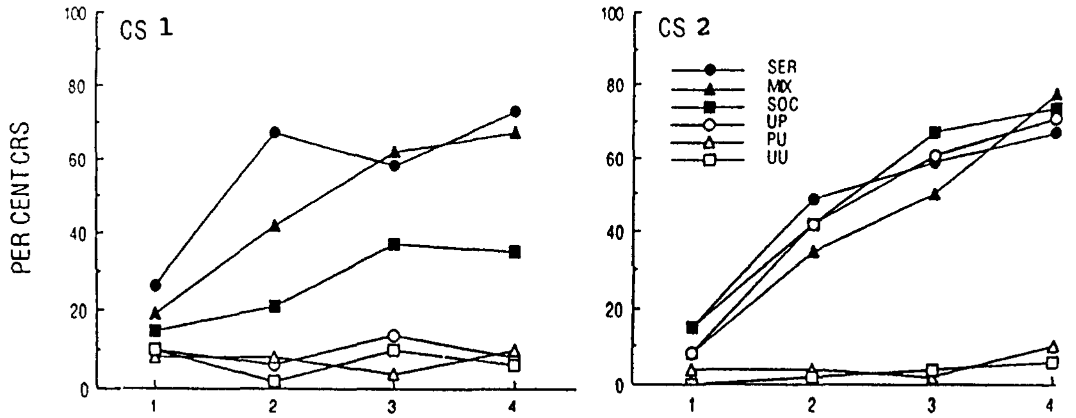

FIG 4. The mean percentage of CRs to CS1 (left-hand panel) and CS2 (right-hand panel) test trials over four-day blocks, for all six groups in Experiment 3.

Groups SOC, MIX, SER, and UP all showed similar CR acquisition curves, rising to 70% CRs by the end of the experiment. On the other hand, the groups that received unpaired presentations of CS2 and the US—Groups PU and UU—showed negligible responding, generally less than 5%. In agreement with these observations, an analysis of variance yielded significant effects of Groups, $F(5,66) = 13.25, p < .01$, and Groups X Blocks, $F(15, 198) = 4.84, p < .01$. Subsequent Tukey *hsd* comparisons for the last block of training confirmed that responding to CS2 in Groups SOC, MIX, SER, and UP was significantly greater than in Groups PU and UU, critical difference (6, 198, a = .05) = 25%. Any other apparent differences failed to attain significance.

Discussion

The major findings of the present experiment were (a) sustained and substantial second-order conditioning occurred; (b) second-order conditioning yielded only half as many CRs to CS1 as produced by serial compound conditioning; and, (c) CR acquisition to CS1 under serial compound conditioning (Group SER) is relatively robust, showing only small, nonsignificant reductions when nonreinforced CS1-CS2 presentations (Group MIX) were interspersed among CS1-CS2-US serial compound conditioning trials.

In attempting to identify the sources of divergence between serial compound and second-order conditioning, comparisons of Group MIX to Groups SER and SOC revealed that nonreinforced CS1-CS2 presentations had little detrimental effect on CS1 or CS2 responding. With reference to Group SER, Group MIX was a 50% partial reinforcement procedure in which the number of reinforced CS1-CS2-US presentations was equated to Group SER. From this perspective, the apparent failure of 50% partial reinforcement to dampen the rate of CR acquisition to the first-order stimulus, CS2, agrees with the previous finding in the rabbit NMR preparation (Gormezano & Coleman, 1975) that a 50% and 100% reinforcement schedule produced the same asymptotic levels of performance.

General Discussion and Conclusions

The present experiments were conducted in order to more completely delineate the determinants of second-order conditioning and to provide estimates of the strength of associative transfer between two CSs. Our main findings were: (a) the rate and level of second-order conditioning were inversely related to CS1-CS2 interval up to 8,400 ms; (b) second-order conditioning was an inverted-U shaped function of the number of CS1-CS2 trials; and, (c) CR acquisition to CS1 in second-order conditioning was about half the strength of that achieved in a reinforced serial compound or in a mixed procedure of second-order and serial compound conditioning trials. These principle findings still leave open the question of whether (a) associative transfer produced relatively weak excitatory conditioning relative to associations based on CS-US pairings, or (b) associative transfer is masked in second-order conditioning by the intrusion of extinctive and/or conditioned inhibitory processes. Accordingly, to complicate the task of delineating the determinants of second-order conditioning, there remains the logical and plausible possibility that both hypotheses may be true.

The present experiments offer mixed evidence on the efficiency of associative transfer. On the one hand, responding to the second-order stimulus, CS1, was always weaker than responding to the first-order stimulus, CS2, which may, however, only reflect a lag in the transfer process. Furthermore, if associative transfer were to produce strong excitatory conditioning of CS1, then the addition of CS1-CS2 trials to serial compound trials in Group MIX of Experiment 3 should have enhanced CR acquisition to CS1. In fact, Group MIX showed slightly, albeit, nonsignificantly, slower CR acquisition to CS1 than did the serial compound group, Group SER. On the other hand, the long tail on the CS1-CS2 interval function suggests that associative transfer is reasonably robust over a rather extended interval. Interestingly, as regards the role of inhibitory processes, the present experimental evidence is more definitive. In Group MIX of Experiment 3, the partial reinforcement of CS1-CS2 had only a slight and nonsignificant depressive effect on responding to CS1 compared to the continuous reinforcement of CS1-CS2 in Group SER. However, much lower responding was seen in Group SOC, which differed from Group MIX only in the absence of CS1 in advance of CS2 on reinforced trials. This manipulation, however, meant that neither CS1 nor the CS1-CS2 compound was ever reinforced by the US. Although some of the loss in CR acquisition to CS1 may be attributed to the absence of direct CS1-US trace conditioning, previous findings indicate that direct conditioning contributes only modestly at the intervals used in Experiment 3 and not at all at longer intervals (Kehoe, et al., 1979, 1981; Kehoe & Morrow, 1984). Accordingly, to the extent that direct conditioning can be discounted, the difference between Group MIX and SOC suggests a considerable intrusion by inhibitory processes into second-order conditioning.

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