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Perseveration of associative strength in rabbit nictitating membrane response conditioning following ISI shifts

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The present experiment determined whether associative strength based upon 15 CS-US pairings at various interstimulus intervals (ISIs) could survive shifts of the ISI to influence the subsequent acquisition of the nictitating membrane (NM) CR. The choice of 15 preshift pairings was based upon previous work, which had shown that this training level produced substantial associative strength without NM CR acquisition. Consequently, this experiment, by shifting the ISI before the beginning of CR acquisition, served as an extension to traditional ISI-shift studies that have imposed the manipulation after CR acquisition. The findings of the experiment indicated that 15 preshift pairings in Stage 1 at ISIs from 250 to 4,000 msec were as effective as 15 pairings at a 500-msec ISI in determining the number of trials to the first NM CR in Stage 2 in which the training ISI was 500 msec. Moreover, 15 pairings in Stage 1 at ISIs from 250 to 2,000 msec were equivalent to 15 pairings at 500 msec in controlling the number of trials to 10 successive NM CRs in Stage 2. These outcomes demonstrate that, within a large ISI range, the associative strength based upon relatively few pairings is preserved despite various shifts of the ISI. Therefore, these results suggest that the reductions in CR performance, which have been consistently observed in traditional studies following ISI shifts, are not due to the loss of associative strength.

Investigators have consistently found that shifts in the interstimulus interval (ISI) reduce CR performance (e.g., Coleman & Gormezano, 1971; Ebel & Prokasy, 1963; Prokasy & Papsdorf, 1965; Wickens, Nield, Tuber, & Wickens, 1969). For example, in nictitating-membrane-response (NMR) conditioning, Coleman and Gormezano (1971) shifted the ISI from 200 to 700 msec and from 700 to 200 msec for independent groups. Their results indicated that shifts in the ISI were followed by immediate decrements in NM CR performance. However, with continued training following the ISI shift, NM CR performance rebounded.

Researchers have been divided regarding the explanation of ISI shift effects. Gormezano and his associates (Gormezano, 1972; Millenson, Kehoe, & Gormezano, 1977) have relied upon Hull's (1943) original hypothesis of a molar stimulus trace to account for ISI shift effects. Gormezano's position asserts that the CS generates a characteristic sensory representation whose energy levels correspond to the shape of the ISI-CR frequency function. Moreover, the amount of associative strength produced by training trials is governed by the amplitude of the CS at the moment of US delivery. If the ISI is suddenly shifted, drops in CR performance are expected due to the lack of associative strength on the CS trace at the new point of US delivery. As associative strength grows at the postshift CS trace value, CR performance returns. By contrast, Prokasy and his colleagues (Ebel & Prokasy, 1963; Prokasy, 1965; Prokasy, Ebel, & Thompson, 1963) have preferred a response shaping theory to explain the effects of ISI shifts. According to the response shaping position (e.g., Kimmel & Burns, 1975; Martin & Levey, 1969; Prokasy, 1965), the temporal overlap of the CR and the US is necessary to bring about reinforcement-sustaining conditioned responding. In classical aversive conditioning, reinforcement may be produced by the ability of the CR to retard the noxious properties of the US; and, in classical appetitive conditioning, reinforcement may stem from the CR enhancing the reward value of the US. Consequently, if the ISI is suddenly shifted, the source of reinforcement for CR performance is disrupted until the CR-US overlap condition is restored.

The traditional studies of ISI shift effects have introduced the manipulation after CR acquisition. The present experiment attempted to extend the information concerning ISI shift effects by imposing the manipulation before the establishment of the CR. Our earlier work (Scavio, Ross, & McLeod, 1983) has demonstrated that 15 CS-US pairings were sufficient to produce substantial associative strength, even though NM CRs did not develop. Therefore, 15 pairings were used as the

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training level preceding shifts of the ISI. Specifically, in Stage 1 of this study, independent groups of rabbits were given the 15 pairings at the following ISIs: 0, 200, 250, 500, 750, 1,000, 1,500, 2,000, and 4,000 msec. These values were selected because they are in the domain of the ISI-CR frequency function for NMR conditioning (Schneiderman & Gormezano, 1964; Smith, Coleman, & Gormezano, 1969). In Stage 2, all groups resumed training by receiving 320 pairings at a 500-msec ISI. The rate of NM CR acquisition in the second stage, relative to control group performance, should reveal whether the consequences of the first-stage training survived the ISI shifts. According to the CS trace theory (Gormezano, 1972), shifts of the ISI, by changing the sensory representation of the CS, should produce generalization decrements in associative strength. Therefore, relative to the no-shift control condition, shifts in the ISI between Stages 1 and 2 should retard the development of the NM CR. However, in his response shaping theory, Prokasy (1965) considers that the CS-US association is established before the CS-US reinforcement process develops. If this association is resilient to the effects of ISI shifts, then Stage 1 training may facilitate NM CR acquisition in Stage 2 independent of the initial ISI provided that Stage 1 is limited to preclude the emergence of NM CRs.

METHOD

Subjects

The subjects were 144 male and female albino (New Zealand) rabbits. The average age and weight of the rabbits were 90 days and 2.30 kg.

Apparatus

The fabrication of the conditioning apparatus followed Gormezano's (1966) specifications. Six conditioning chambers were constructed from legal-sized filing cabinets. A stimulus panel, containing a pair of 24-V dc, 10-W lamps for illumination and an audio speaker for transmitting the CS, was attached to the interior front side of each chamber. Plexiglas boxes, with variable-position backplates and yoke collars, were used to restrain the rabbits while they were in the conditioning chambers.

Movement of the nictitating membrane was measured by a microtorque potentiometer that was positioned on a muzzle securely locked to a ring looped over the pinnae. The potentiometer contacted the nictitating membrane in the following manner. A counterbalanced wire lever, attached to the potentiometer's rotary shaft, issued a length of silk thread that was tied to a small metal hook. In turn, the hook was connected to a suture incisioned into the lateral edge of the right nictitating membrane. Movements of the membrane were transduced by the potentiometer into linear voltage changes that were first amplified and then recorded by the analog-to-digital converter of a PDP-12 computer.

The CS was a 1000-Hz tone of 86 dB (re: 20 $\mu N/m^2$) superimposed on a 72-dB white-noise field. The US was a 50-msec, 4-mA, 60-Hz electrical shock delivered through stainless steel wound clips embedded in the skin 10 mm apart and 15 mm behind the right eye. Finally, the PDP-12 computer was programmed to present the CS and US as well as record NMRs (Bissell & Scavio, 1974).

Procedure

Two days after arriving from the supplier, each rabbit received a suture in the right nictitating membrane made with 00 monofilament surgical thread. After 2 more days, each rabbit was fitted with a potentiometer and placed in the conditioning apparatus for a 40-min adaptation session that was free of CS and US presentations. On the following day, Stage 1 was initiated by randomly assigning 12 subjects to each of 12 groups. Nine of the groups were given 15 CS-US pairings equally divided over three daily sessions (i.e., 5 daily pairings). However, each of the 9 groups received the pairings at different ISIs selected from the following values: 0, 200, 250, 500, 750, 1,000, 1,500, 2,000, and 4,000 msec. The 3 remaining groups served as controls and received the following treatments. Group N, which did not receive any CS or US presentations, was placed in the conditioning apparatus on each of the 3 successive days of Stage 1 for time periods lasting the length of the training sessions for the groups that received 15 CS-US pairings. Group U was given 5 presentations each of the CS and US on every Stage 1 day according to an "explicitly unpaired" schedule (Rescorla, 1967). Group R obtained 5 presentations each of the CS and US on every Stage 1 day according to the "truly random" procedure (Rescorla, 1967). Since the truly random procedure requires that the CS and US be independently programmed, 2 forward pairings occurred on the 1st and 3rd days of Stage 1 at ISIs of 347 and 1,620 msec, respectively. Also, 1 backward pairing occurred on the 2nd day at an ISI of 604 msec. The duration of the CS in Stage 1 was 250 msec for the groups receiving the 15 pairings at the 0-, 200-, and 250-msec ISIs. For the remaining pairing groups, the CS duration matched the length of the ISI. For Groups U and R, the CS duration was 500 msec. Finally, the intertrial intervals for the groups receiving the 15 pairings in Stage 1 were randomized at values of 50 and 70 sec with a mean of 60 sec. For Group U, the interevent intervals, separating unpaired CS and US presentations, were randomized at values of 25 and 35 sec with a mean of 30 sec. For Group R, the interevent intervals between CS and US presentations were randomized throughout a range of values. However, the length of the daily sessions for Group R and the other groups was the same.

Stage 2 began on the next day following the completion of Stage 1. In the second stage, all groups were given 80 CS-US pairings on each of 4 consecutive days. The CS duration and the ISI were both 500 msec on all pairings in the second stage. Finally, the intertrial intervals for all groups averaged 50 and 70 sec, with a mean of 60 sec.

Extensions of the NMR of at least .5 mm during the ISIs on the forward pairings of Stages 1 and 2 were scored as CRs. For Groups U and R in Stage 1, NMR extensions meeting the .5-mm criterion during presentations of the CS were treated as CRs. For Group N in Stage 1, NMR activity was monitored at times corresponding to the deliveries of the unpaired CS given to Group U. Criterion extensions of the NMR during these observation intervals provided an estimate of base-level activity.

RESULTS

The percentage of NM CRs over each Stage 1 day was compiled for all groups. Since none of the groups exceeded the daily 3% NMR base-level shown by Group N, no NM CR acquisition was apparent. This observation was substantiated by an analysis of variance which found no differences for groups ($F < 1.00$) and for days ($F < 1.00$).

The transfer effects of Stage 1 training were assessed upon the number of trials in Stage 2 required for the first NM CR and 10 successive NM CRs. The small, left panel of Figure 1 shows that Control Groups U, N, and R averaged between 51 and 62 pairings in Stage 2 to initiate the first CR. In contrast, the large, right panel of the figure indicates that 15 pairings in Stage 1, at

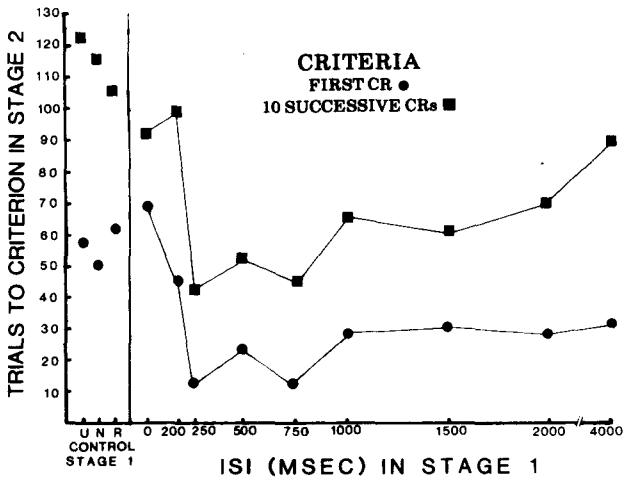

Figure 1. Mean number of trials to the first CR and 10 successive CRs in Stage 2 as a function of the control procedures (left panel) and the training ISI (right panel) in Stage 1.

ISIs ranging from 250 to 4,000 msec, were as effective as 15 pairings at the 500-msec ISI in reducing the number of trials to the first CR in Stage 2 (where the ISI was 500 msec). However, the 15 pairings in Stage 1 at the 0- and 200-msec ISIs did not contribute any positive transfer effects to the establishment of the first CR in Stage 2.

An analysis of variance on the number of trials in Stage 2 for the first NM CR indicated significant group differences [$F(11,132) = 4.19$, $p < .01$]. A subsequent Newman-Keuls test revealed that the means for Groups N ($\bar{X} = 51$), U ($\bar{X} = 57$), and R ($\bar{X} = 62$), as well as for groups trained in Stage 1 at the 0-msec ($\bar{X} = 67$) and 200-msec ($\bar{X} = 47$) ISIs, were not significantly different (ps $> .05$). However, all of these groups required significantly more (ps $< .05$) trials in Stage 2 for the first CR than the groups trained at the 250- ($\bar{X} = 12$), 500- ($\bar{X} = 24$), 750- ($\bar{X} = 11$), 1,000- ($\bar{X} = 28$), 1,500- ($\bar{X} = 30$), 2,000- ($\bar{X} = 29$), and 4,000-msec ($\bar{X} = 33$) ISIs in Stage 1. Finally, no significant differences (ps $> .05$) occurred among the groups trained at the ISIs from 250 to 4,000 msec in the first stage.

Returning to Figure 1, the left panel shows that Control Groups U, N, and R averaged between 106 and 122 pairings in the second stage before the development of 10 successive CRs. (The number of trials to 10 successive CRs serves as a measure of the training requirements for asymptotic conditioned performance.) In comparison, the right panel indicates that the 15 pairings in Stage 1 at ISIs from 250 to 2,000 msec were as effective as 15 pairings at 500 msec in determining the NM CR asymptote in Stage 2. However, the first-stage pairings at the 0-, 200-, and 4,000-msec ISIs were not effective for establishing asymptotic NM CR performance in the second stage.

An analysis of variance on the number of trials in Stage 2 for 10 successive NM CRs revealed significant group differences [$F(11,132) = 5.25$, $p < .01$]. A subsequent Newman-Keuls test gave the following comparisons. Groups R ($\bar{X} = 106$), N ($\bar{X} = 115$), and U ($\bar{X} = 122$), not differing from one another (ps $> .05$), were also similar (ps $> .05$) to the groups receiving first-stage training at the 0- ($\bar{X} = 92$), 200- ($\bar{X} = 99$), and 4,000-msec ($\bar{X} = 90$) ISIs. However, all of these groups required significantly more (ps $< .05$) pairings in Stage 2 than did the groups trained in Stage 1 at the 250- ($\bar{X} = 44$), 500- ($\bar{X} = 52$), and 750-msec ($\bar{X} = 47$) ISIs. Finally, pairings in Stage 1 at the ISIs of 250, 500, 750, 1,000 ($\bar{X} = 64$), 1,500 ($\bar{X} = 61$), and 2,000 msec ($\bar{X} = 68$) were equivalent (ps $> .05$) in promoting asymptotic CR performance in Stage 2.

DISCUSSION

Although 15 CS-US pairings in Stage 1 were insufficient for the occurrence of NM CRs, this training level produced enough associative strength capable of surviving shifts in the ISI to initiate NM CR acquisition in Stage 2. Specifically, the ISI was shifted from Stage 1 values in a range from 250 and 4,000 msec to 500 msec in Stage 2 with no apparent retardation in the onset of NM CR acquisition. Also, the number of trials for the establishment of asymptotic NM CR performance in Stage 2 was the same whether the 15 pairings in Stage 1 were given at ISIs from 250 to 2,000 msec or at 500 msec.

The present outcomes are relevant for the interpretation of traditional ISI shift studies, which have shown that the manipulation produces deficits in previously established CR performance (e.g., Coleman & Gormezano, 1971; Ebel & Prokasy, 1963; Prokasy & Papsdorf, 1965; Wickens et al., 1969). Our findings indicate that associative strength, based upon relatively few trials, survives ISI shifts as large as 3,500 msec to aid the occurrence of the first NM CR and as large as 1,500 msec to promote the occurrence of asymptotic NM CR performance. Therefore, reductions in NM CR frequency, which are known to exist after ISI shifts as small as 500 msec (Coleman & Gormezano, 1971), appear to be caused by a factor other than the loss of associative strength. Although the data offer no direct evidence that a reinforcement process is disrupted by ISI shifts, our outcomes are consistent with certain features of Prokasy's (1965) response shaping theory. As noted in the introduction, Prokasy (1965) has proposed that CS-US pairings produce associative strength before the appearance of the overt CR. The present indications that associative strength, based upon an insufficient number of pairings for NM CR elaboration, can nevertheless withstand ISI shifts to promote subsequent NM CR acquisition are consistent with Prokasy's assessment.

It is interesting to compare the shapes of the functions for the trials with the first NM CR and 10 successive NM CRs depicted in Figure 1. The functions have the same initial descending phase in that preshift training at the 0- and 200-msec ISIs was ineffective for subsequent NM CR performance. However, the function for

the first NM CR has a broader minima, extending to 4,000 msec. By contrast, the function for 10 successive NM CRs has a gradual subsiding phase marked by the inability of the preshift training at the 4,000-msec ISI to influence the establishment of the conditioned performance asymptote. The failure of training at the zero and 200-msec ISIs to control subsequent NM CR performance may have been due to the lack of associative strength. In this regard, the ISI is known to determine the rate of associative growth in NMR conditioning (Schneiderman & Gormezano, 1964; Smith et al., 1969). The paradoxical effects of preshift training at the 4,000-msec ISI are harder to explain. Since the occurrence of the first NM CR was facilitated, preshift training at the 4,000-msec ISI apparently contributed associative effects. However, the resulting associative bond developed by preshift training at the 4,000-msec ISI may not have included the necessary properties required for consistent NM CR performance.

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