Serial order of conditional stimuli as a discriminative cue for Pavlovian conditioning

Robin A. Murphy a, *, Esther Mondragón b, Victoria A. Murphy b, Nathalie Fouquet a

a Department of Psychology, University College London, Gower Street, London WC1E 6BT, UK
b Department of Educational Studies, Oxford University, UK

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Abstract

The serial order in which events occur can be a signal for different outcomes and therefore might be a determinant of how an animal should respond. In this report, we propose a novel design for studying serial order learning in Pavlovian conditioning. In both Experiments 1a and 1b, hungry rats were trained with successively presented pairs of auditory and visual stimuli (e.g., A → B) using four different stimuli (A–D). Four orders were paired with food (A → B, B → C, C → D, D → A) while the reversals were extinguished (B → A, C → B, D → C, A → D). An analysis of responding from the second element of each pair showed that the rats discriminated trial types that preceded food from those that did not. A replication of the effect using a completely counterbalanced design is described in Experiment 1b. These results suggest that rats can use the serial or temporal order of two sequentially presented non-overlapping elements as the basis for discrimination. Two associative accounts are suggested as possible mechanisms for solving the discrimination.

Keywords: Pavlovian conditioning; Serial order; Discrimination; Sequences

1. Introduction

The temporal or serial order in which stimuli occur can have important implications for predictive behaviour. Sequential learning has been defined as the ability to encode the temporal order in which stimuli occur (Conway and Christiansen, 2001). Examples of naturally occurring serially ordered stimuli are found in both auditory (e.g., animal communication) and visual contexts. Some previous work has claimed to find evidence of encoding of serial order in animals (e.g., Weissman et al., 1980). However, much of this work might be explained by associative learning that does not encode explicit order information. In this paper, we propose a novel experimental design to identify temporal order learning within Pavlovian conditioning.

There is good evidence that within a standard CS-US conditioning procedure the order in which these two events are presented influences subsequent behaviour. Forward pairing of an initially neutral conditioned stimulus (CS) and the unconditioned stimulus (US) results in a conditioned response. Backward pairing of the two events also produces learning but of a somewhat different nature, suggesting the importance of the temporal order of the stimuli. For example, Moscovitch and LoLordo (1968) showed that unlike forward pairings of the CS and US that produces excitatory conditioning, presenting
the CS following the US resulted in inhibitory conditioning. Given the sequential nature of the stimuli in many learning procedures, it is somewhat surprising that many associative theories of conditioning fail to integrate temporal information in the associative structure. One possible reason for this neglect may be the fact that standard associative mechanisms can explain most conditioning phenomena without requiring an explicit coding of the temporal information.

There is actually relatively little research directly studying discrimination of sequentially presented non-overlapping CSs. The work that has been conducted has yielded results consistent with the hypothesis that animals can acquire temporal order information during Pavlovian conditioning. Seger and Scheuer (1977) showed that responding was greater to the second CS of two successively presented CSs that preceded a US (A → B → US) than to that same stimulus when it was presented at the beginning of the extinguished trial (B → A → no US). The solution of this simple discrimination, however, does not provide convincing evidence that the animals have encoded or represented the temporal order of the two cues. As Weisman et al. (1980) have suggested, they might simply use a recency or primacy strategy. For example, the animals may simply have learnt to respond at the end of the compound, and therefore any comparison of responding to B alone would suggest that animals had solved the discrimination. Solving this discrimination does involve encoding some aspect of the temporal delay between the beginning of the trial and the termination of the second CS. But it does not require that the animals represent or code the temporal order of A and B, although their performance might be consistent with this hypothesis. A comparable account has been used by Terrace (1986) to explain a similar discrimination with pigeons.

Weisman et al. (1980) describe a set of more elaborate experimental designs used to test whether pigeons could represent the order of two-event sequences. They used a conditioned pecking procedure with pigeons in three experiments. Pigeons were reinforced with grain for pecking a key following the presentation of a two-element stimulus sequence. In their Experiment 2, they showed that pigeons responded more at the end of a reinforced sequence (A → B⁺) than after the non-reinforced sequences involving all other combinations of A and B (A → A⁻, B → A⁻ or B → B⁻) and combinations of A and B with a novel third stimulus C (A → C⁻, B → C⁻, C → A⁻, C → B⁻ or C → C⁻). They proposed that the pigeons could only solve this discrimination by representing the order in which the stimuli were presented, A followed by B, and associating this order with food. However, a different strategy that does not involve temporal coding might have been possible. The highest rates of responding were found when B was preceded by A (i.e., the reinforced sequence), but there was also strong responding whenever B occurred as the second stimulus, regardless of the preceding element (B → B, X → B). This pattern might suggest a strong association between the element B and the US particularly if it occurred later in the trial pair, much like the account described earlier for the experiment by Seger and Scheuer (1977). There was further evidence that response rates were higher to the specific reversal of the A–B sequence (i.e., when B was followed by A). This behaviour might suggest that the sequential presentation may have established a configural cue without representing any temporal coding of the order of the stimuli. Even though the stimuli were non-overlapping it has been suggested that serial presentation of stimuli may foster configural learning (e.g., Holland, 1983).

Given their evidence, it is possible that two associations with the US were formed; these two associations may sum their effects on behavior, resulting in the successful discrimination. First, an association of the configuration formed by A and B (the two elements that were reinforced) with the US could have formed. Secondly, an association between the occurrence of B and the US could have emerged. Since all tests of discrimination learning involve analysing behaviour during the period following the second element, any sequence either containing the AB configuration regardless of temporal order, or B as the second element, would also elicit relatively high rates of responding. Such a mechanism for solving the temporal order discrimination is entirely consistent with the pattern of responding observed and is also a relatively simple way to solve the discrimination without encoding the temporal order. Furthermore, summation of associative strengths is a major tenet of some associative theories (e.g., Rescorla and Wagner, 1972) and has been reported elsewhere (e.g., Murphy et al., 2001; Rescorla, 1997).
In their Experiment 3, Weisman et al. (1980) extended their findings using a conditional discrimination paradigm in which three element sequences were used. Forward and backward presentations of A and B (A → B and B → A, respectively) were reinforced depending upon the identity, C or D, of a subsequent third stimulus. C and D were presented simultaneously on two response keys. Pecking to stimulus C, for example, was reinforced following the sequence A → B and D following the sequence B → A, but all other combinations were extinguished (AAC, AAD, ABD, BBC, BBD, BAC). The animals were able to solve this discrimination and chose the correct key. That is, they responded differentially to C and D depending upon the order in which A and B had been presented. This result, however, can also be explained on the basis of the summation of two associations with the US involving configural cues. If the order in which the stimuli were presented is ignored, there were three different temporally contiguous pairs of stimuli in the two reinforced sequences: A and B, B and C, and A and D. Each of these pairs may have generated configural cues that became associated with the US without encoding temporal information. Both reinforced triplets (ABC and BAD) contain two of these configurations while the non-reinforced compounds contain only one (AAD, ABD, BBC, BAC) or none (AAC, BBD). If the associations that form between the compounds and the US sum, then the high level of responding to ABC and BAD would be easily predicted. Interestingly, this hypothesis also predicts that responding should be lowest during AAC and BBD, the two triplets that contain neither of the two-element compounds present in the reinforced sequences and an intermediate level of responding to the other non-reinforced sequences. The figures presented in the original paper are consistent with these predictions. One interpretation of performance during these experiments then is that animals form configural cues for non-overlapping sequentially presented stimuli and that these cues become associated with the US.

2. Experiments 1a and 1b

We propose an alternative experimental design to assess serial-order learning during Pavlovian conditioning that cannot be solved simply on the basis of the summation of configural associations formed between pairs of stimuli. This design involves presenting pairs of stimuli, both reinforcing and extinguishing each stimulus an equivalent number of times and having each element presented in the first and the second positions the same number of times. The design involved training with four different elements (A–D) arranged to generate four unique stimulus pairs, presented in two orders producing eight stimulus compounds. Four compounds resulted in food (A → B, B → C, C → D, D → A) and the reversals of these were extinguished (B → A, C → B, D → C, A → D). In addition, to minimise differences in generalisation between pairs of stimuli, each pair consisted of an auditory stimulus and a visual stimulus. Thus, all four pairs consisted of the same combination of stimulus modality, reducing the possibility that any cross-modal differences between compounds might aid the discrimination. Finally, this design also reduces the possibility of generalized occasion setting as reported by Honey and Watt (1998, Experiment 1). Each stimulus (e.g., A) precedes both a reinforced stimulus (e.g., B) and a non-reinforced stimulus (e.g., D; i.e. A → B and A → D−). Each stimulus (e.g., A) has a corresponding stimulus (e.g., C) that precedes the same two stimuli (B, D); however, C signals the reverse reinforcement contingencies (B−, D+) from that of A. Therefore, it is unlikely that learning about A and its two associations would result in some form of acquired equivalence between A and its corresponding stimulus C (e.g., Honey and Watt, 1998) that might facilitate discrimination of the C sequences.

2.1. Experiment 1a

2.1.1. Method

2.1.1.1. Subjects. The subjects were eight naïve male Wistar-derived rats (Rattus norvegicus) obtained from the breeding colony at the University of Hertfordshire. They started the experiment approximately 90 days old and weighing between 400 and 450 g. Their weights were reduced to 85% of their free-feeding weight before the start of the experiment and remained at this level for the duration of the experiment. They were housed in pairs and in a room lit on a 12:12-h light:dark cycle.
2.1.1.2. Apparatus. Conditioning for both experiments took place in eight identical conditioning chambers (MED Associates, East Fairfield, VT.) with internal dimensions of 32 cm wide, 21 cm high and 26 cm deep. The chambers were housed in sound and light attenuating cubicles with background noise produced by ventilation fans (≈80 dB). In the middle of one wall was a food magazine tray into which 45 mg food pellets (Noyes, Formula A) could be delivered. Head entries into the food tray were recorded by a single optical integrated circuit sensor and infrared LED; where breaking the beam counted as a single entry. There were four conditional stimuli: two auditory (A and C) and two visual (B and D). A tone (stimulus A; not sinusoidal) with a fundamental frequency of 2.8 kHz (86–87 dB SPL) was generated by a piezo electric crystal (KPE-350; Farnell Electronics, Leeds, UK). The same tone was used for stimulus C but pulsed at five times a second. For the light stimulus (stimulus B), the 28-V stimulus light located above the food magazine was illuminated or darkness (stimulus D) was produced by turning off the houselight. Between trials, the chambers were illuminated by a dim houselight on the wall opposite the food tray.

2.1.1.3. Procedure. All animals were trained with a single 30-min session of magazine training during which free pellets were delivered according to a variable interval 60-s schedule. Each conditioning session involved presenting 80 trials on a variable time 68-s schedule. There were 10 presentations of each of the four reinforced pairs (A → B, B → C, C → D, D → A) and the reversals of these that were extinguished (B → A, C → B, D → C, A → D). Each trial comprised a 10-s presentation of S1 followed by a 1-s gap followed by onset of a 10-s presentation of S2. Food pellets were programmed to coincide with the offset of S2 at the end of reinforced trials. The four stimulus compounds (S1 → S2) were assigned to ensure that each sequence included one auditory and one visual stimulus. The four sequences that were followed by food were Tone–Light, Light–Pulsing Tone, Dark–Tone and Pulsing Tone–Dark, and the reverse order of these pairs were extinguished. Training continued for 21 consecutive sessions. Magazine tray entries were recorded during the first and second element of each sequence and are reported as the number of times that the animal introduced its head in the tray during the intervals. A 0.05 rejection criterion was used for all statistical tests. Effect sizes are reported using the conservative partial omega coefficient as described by Keppel and Wickens (2004, pp. 360–362). They recommend 0.01, 0.06 and 0.15 as the approximate boundaries for small, medium and large effects. These values represent estimates of the proportion of the variability in the population. Effect size estimates are included to demonstrate the strength of reported statistically reliable effects and conversely the weakness of non-reliable differences independent of sample size.

2.1.2. Results and discussion

The dependent measure was the rates of head entries in the magazine tray per minute analysed in three session blocks. Initially, head entries were distributed throughout the session but as training continued responding occurred more frequently during the second element S2. Consistent with the hypothesis that rats would learn which two-element sequences were paired with the food, responding was greatest during S2 on the reinforced sequences. The following analysis supports these assertions.

Discrimination was assessed by analysis of responses during S2 of each sequence. During S1, the rats could not predict whether the trial was to be reinforced or not, and accordingly, there was no reliable difference in response rates during S1 on reinforced compared with non-reinforced trials. The mean difference on the seven blocks of three sessions of training varied little from 0 (−0.19, 0.04, 0.63, −1.61, −1.58, 0.49, −1.11, respectively for blocks 1–7). An ANOVA testing for the factor blocks failed to find any reliable effect, \( F(6,42) = 1.52, \) partial \( \omega^2 = 0.053 \). However, there was a reliable difference between responding on reinforced and non-reinforced trials during S2. Since the same four stimuli comprised S1 and S2 and were present on both reinforced and non-reinforced trial sequences, and since responses were recorded before the US was delivered, increased responding on reinforced relative to non-reinforced S2 reflects the extent of discrimination learning. Fig. 1 presents responding during S2 as a difference score reflecting the difference in responding to A–D on reinforced trials (S2+) from responding to the same four elements on non-reinforced trials (S2−). A repeated measures ANOVA across the seven blocks of three sessions found a reliable main effect of blocks, \( F(6,42) = 3.84, \)
Responses per minute ($S_2^+ - S_2^-$)

-1.5  -1  -0.5  0  0.5  1  1.5  2  2.5  3  3.5

Blocks of 3 Sessions

Fig. 1. The mean head entry response difference scores ($S_2^+ - S_2^-$) reflecting acquisition of the discrimination during training in blocks of three sessions in Experiment 1a.

partial $\omega^2 = 0.233$. This result supports the observation that the difference scores increased over the training blocks.

The top panel of Fig. 2 shows the levels of responding during reinforced and non-reinforced trials separately for the last block of training. This difference shows that rats responded more to A–D when they were part of the reinforced sequence than when they were part of the non-reinforced sequence. A two-factor ANOVA for trial type ($S_2^+ - S_2^-$) and sequence (A–B, B–C, C–D and D–A) found a main effect for the difference between $S_2^+$ and $S_2^-$, $F(1,7) = 15.57$, partial $\omega^2 = 0.477$, and a main effect for sequence indicating that there were differences in rates of responding to the four sequences $F(3,21) = 4.76$, partial $\omega^2 = 0.260$, but importantly, there was no interaction between these two factors $F(3,21) = 1.30$, partial $\omega^2 = 0.014$, suggesting that the difference was consistent across the four sequences.

The results of this experiment quite clearly demonstrate that rats can discriminate between instances of a stimulus solely according to whether it was part of a reinforced or non-reinforced pair. The effect size estimates confirm that the discrimination effect was quite large. These estimates are also useful for confirming that, in spite of the relatively small number of subjects, the non-reliable interaction of trial type and sequence is associated with a small effect. This is important as it indicates that the discrimination is not based on only some of the pairs. The order in which the two stimuli
Experiment 1a

![Fig. 2. The mean head entry responses during the second element for reinforced (S₂⁺) and non-reinforced (S₂⁻) trials on the final block of training in Experiment 1a (top panel) and Experiment 1b (bottom panel).]

Stimulus type

Experiment 1b

of the pair were presented acted as a discriminative cue for reinforcement.

Although the same four stimuli were present on both reinforced and non-reinforced trials, the specific orders that were assigned as reinforced were not counterbalanced. For example, Tone followed by Light was always reinforced and the reverse was extinguished. It is possible then that the assignment of reinforcement to the orders may mask some bias to learn about these specific orders, although given the complex nature of the discrimination this is highly unlikely. Nonetheless it is not impossible, and therefore to rule out this explanation, a second version of this experiment was carried out with half the animals receiving the same treatments as Experiment 1a and half the animals receiving the same stimulus pairs but in a counterbalanced order. This design provides both a replication of Experiment 1a and a test of the reverse orders.

In addition, in an attempt to increase the size of the discrimination, two changes were made to the training procedure. The inter-trial-interval (ITI) was lengthened and the duration of the stimuli was shortened from 10 to 6 s. Increasing the ITI duration might be expected to reduce interference between trials, and thereby enhance the discrimination of reinforced from non-reinforced trials. Decreasing the stimulus duration was predicted to shorten the overall trial length, and thereby reduce the chance of forgetting which stimulus was presented first during the course of the trial.

2.2. Experiment 1b

2.2.1. Method

2.2.1.1. Subjects. 16 male Hooded Lister rats were obtained from a local breeder (Charles River). They were housed and maintained under the same conditions as in Experiment 1a.

2.2.1.2. Apparatus. The same chambers and stimuli were used except that the pulsing tone was replaced with clicker. The click was produced with an electro-mechanical relay attached to the outside wall of the conditioning chamber; when operated at 7.5 Hz, it produced an audible click and vibration to the chamber.

2.2.1.3. Procedure. As in the previous experiment, one session of magazine training was conducted prior to the conditioning sessions. The procedure involved the same training parameters except that the mean ITI for the variable time schedule was 123 s. In addition each element of the pair was only presented for 6 s, with a 1-s gap between stimuli. Another difference between the two versions of the experiment was that in Experiment 1b, the sequences were counterbalanced.
with respect to order and reinforcement. Half the animals received Tone → Light, Light → Click, Dark → Tone and Click → Dark as the order that were paired with the US, while the other half received the US for the reversed orders (i.e., Light → Tone, Click → Light, Tone → Dark, Dark → Click). In spite of our attempts to strengthen the discrimination performance, discrimination was weaker following 26 days, and there was no reliable evidence that the rats had solved it. At this point, we reduced the total number of trials per session from 80 to 48 while maintaining the session duration. Almost immediately, discrimination performance improved. Since this change did not involve differential experience with the stimuli themselves the only effect was on the level of performance in each session. We do not report the training data.

2.2.2. Results and Discussion

Consistent with the results of Experiment 1a, after the reduction in the number of trials per session rats demonstrated a higher level of responding to the second element of reinforced sequences than to the second element of non-reinforced sequences. The absolute rates of responding were lower than those in Experiment 1a perhaps reflecting the shorter stimuli durations and/or the counterbalancing. The bottom panel of Fig. 2 presents the rates of responding to S2 from the last block of two sessions, separated by whether it was part of a reinforced or nonreinforced stimulus pair. A two-way ANOVA for trial type (S2+ or S2−) and sequence (A-B, B-C, C-D and D-A) found a main effect for the difference between S2+ and S2−, F(1,15) = 6.09, partial ω² = 0.137, and a main effect for sequence type, indicating that there were differences in rates of responding to the four different elements, F(3,45) = 3.37, partial ω² = 0.100, but importantly, no interaction between the two main effects, F(3,21) = 0.64, was found, partial ω² = 0.00, suggesting that the difference was consistent across the four sequences.

3. General discussion

The results of these two experiments support the conclusion that rats can use the serial order of two element sequences as a discriminative cue for reinforcement. This is not the first demonstration that temporally ordered conditioned stimuli can influence responding (e.g., Weisman et al., 1980). However, our experimental design eliminates an associative explanation based on configural learning that does not require coding the order in which two stimuli are presented.

Associative theories of learning generally assume that following CS-US pairings an association is formed between the CS and US (e.g., Rescorla and Wagner, 1972; Pearce, 1987). With multiple simultaneously presented CSs, these theories assume that configural associations or within-compounds associations are formed. However, with sequentially presented CSs (S1 → S2), the assumption has traditionally been that animals learn individual chains of associations from S1 to S2 and from S2 to the US (Terrace, 1986). Configural models involving the formation of associative units that code the entire stimulus configuration (Pearce, 1987) have successfully accounted for learning in which multiple stimuli are presented simultaneously. However, a purely configural account without temporal order would have trouble explaining the results of our experiments since each stimulus pair that might result in the formation of a configural association was both reinforced and non-reinforced. Furthermore each of the four stimuli used in the experiment immediately preceded the US, and therefore, there was no unique stimulus that consistently preceded the US. To solve the discrimination, animals would have to learn the specific order of each stimulus pair.

Recently, Pearce (1987) has reported a similar issue with respect to spatial order. He has extended his configural model to include units that code the spatial relationship between simultaneously presented cues (George and Pearce, 2001). Spatial units code the relative position in space of stimulus events. A similar strategy that allows the coding of relative temporal position might be used to account for the present data.

Another possible account involves stimulus traces. Wagner and Brandon (2001) have proposed a real-time model of Pavlovian conditioning that predicts configural associations but the model also predicts that these configural representations might contain information about stimulus onset and offset or, more specifically, about the particular level of activation of each stimulus representation. This information...
Alternative explanations originally used to describe occasion setting that do not involve configurational learning could also account for the present data. A simple serial occasion setting procedure involves sequentially presented stimuli in which the first element (the feature) determines responding to the second element (the target) of a two element sequence, (e.g., A → B → US and B → no US). In the negative version of this procedure, the feature will signal that the target will not be followed by the US (e.g., A → B → no US and B → US).

The experimental design reported here can be conceptualised as an occasion setting design in which every stimulus acts as both a feature and a target depending upon the pair within which it is embedded. In this way, the signalling properties of B in A → B → US trials, for example, are determined by the immediately preceding stimulus. The assumption would be that A modulates or facilitates the B → US link. On trials in which B signals, the absence of the US (C → B → no US), C may become associated with a B → no US link (Bonardi and Ward-Robinson, 2001; Honey, 2000), or may inhibit the link between B and the US. (Rescorla, 1985; Holland, 1983; Bouton and Swartzentruber, 1986). This ambiguity in value of B as target, is considered critical in the development of standard occasion settings properties (but see, Bonardi, 1989; Hall and Mondragón, 1998). However, our procedure also requires that in addition to being the target stimulus in some of the conditions, B will also act as a feature facilitating the C → US and inhibiting the A → US association. It would be possible to generalise this account to our discrimination with neutral stimuli. The encoding of temporal information does not necessarily require an isomorphic representation of time but a representation that, in some way or other, reflects the temporal structure of events.

Given the nature of auditory stimuli used in animal communication, it is not surprising that animals have the ability to encode temporal order in some way. Our experiments used both auditory and visual stimuli pairs, suggesting a general ability to learn that temporal order is not restricted to communication but may represent a general cognitive capacity.

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References


