Latency of the two-way avoidance response in rats: inhibition of delay

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Abstract. Avoidance and escape latencies were examined in 24 rats trained in two-way avoidance response to auditory and visual conditioned stimuli (CS). In rats trained with darkness CS the escape latencies decreased within the first 50-trial session but later were stable over the course of nine sessions. Avoidance latencies shortened within sessions as the response-eliciting properties of the conditioned stimuli increased. On the contrary, median daily avoidance latencies lengthened during training. Training with the more salient auditory stimulus resulted in a decrease in the probability of avoidance responses early in the CS-US interval, reflecting the development of inhibition of delay. Acquired inhibition of delay was eliminated by an increase in the fear-inducing properties of situational cues. When presented in a compound, the less salient darkness stimulus was fully overshadowed by the noise stimulus.

Key words: response latency, two-way avoidance, conditioned stimulus modality, inhibition of delay, rat
INTRODUCTION

The superiority of an auditory over a visual warning signal in two-way avoidance learning of rats is well documented. A variety of experimental designs and different measures of dependent variables were used (Anisman and Waller 1973, Satinder 1976, Bignami 1989). Recently it was shown that stimulus modality effects on avoidance performance were combined with pronounced differences in the rate of shuttle responses performed during intertrial intervals (Zielinski et al. 1991). In our experiments rats were permitted to move in any direction during intertrial intervals. A crossing from one compartment to the other was scored as an intertrial response (ITR). In these conditions changes in illumination used as a conditioned stimulus (CS) produced lower avoidance performance and much higher rate of intertrial responses than an onset of a wide band noise CS. When the two stimuli were presented in a compound, both effects of the visual stimulus were completely overshadowed by the noise.

The main source of ITRs during defensive learning is residual fear from a previous trial which summates with fear associated with the experimental context (Zielinski 1993). The stimuli of different modalities differ not only in salience but also in the discriminability between the onset of a stimulus and its termination. We postulated (Zielinski et al. 1991) that the high ITR rate in rats trained with a visual CS was due to insufficient discrimination of the opposite changes in illumination level signalling respectively danger and safe periods in the shuttle box. This notion was supported by the similarity of results obtained in two different groups of rats: one, trained to avoid shock signalled by onset of illumination in the occupied compartment by running to the safe dark compartment, and the other group, trained to run from a dangerous darkened to the safe light compartment. Opposite directions of change were used, but absolute difference in illumination between the warning signal and the intertrial interval was the same in both groups (Zielinski et al. 1991).

The low effectiveness of the visual CS in our experiments may be related to the within-session variability of avoidance performance. In rats trained with the visual CS the level of avoidance responding at the beginning of each successive session was lower than that attained toward the end of the previous training session. In rats trained with the auditory CS insufficient retention of the avoidance response was observed only during the early sessions. The ITR rate displayed a similar increased within-session trend but only when rats were trained with the visual CS (Zielinski et al. 1991).

The aim of the present paper is further examination of the CS modality effects on two-way avoidance learning in rats. The data concerning avoidance performance and intertrial responding in three groups of rats trained with auditory, visual and compound stimuli presented before (Zielinski et al. 1991, Expt. I) will be supplemented here by analysis of avoidance and escape latencies.

METHOD

Apparatus

The shuttle-box apparatus consisted of two identical opaque dark Plexiglas compartments (31 cm long, 18 cm wide, and 29 cm high) separated by an opaque dark insert with a rectangular (7 cm wide, 10 cm high) cutout situated on the grid-floor level permitting passage from one side of the shuttle-box to the other. Each compartment was illuminated by a 5 W lamp mounted centrally just below a movable transparent Plexiglas ceiling. On each wall opposite to the central partition, a 10 cm loudspeaker was mounted outside of the apparatus and 15 cm above the floor. The response of crossing through the opening was recorded by photocells mounted 4 cm to either side of the central partition, 5 cm above the floor level. The floor in each compartment was constructed from 16 stainless steel bars, 0.4 cm in diameter, and located parallel to the central partition 1.5 cm apart from each other.
Subjects and design

The experiment was conducted with 24 adult male Moll-Wistar rats bred in the Institute, experimentally naive, and weighing 320-360 g. Details of maintenance of the rats were published previously (Zielinski et al. 1991).

Prior to avoidance training each rat was habituated to the situational cues of the apparatus for 10 min on two consecutive days. Rats were trained once a day in the morning or early afternoon, according to the same order and about the same time each day. At the beginning of each training session the rat was placed in the left compartment of the shuttle-box, close to and facing the end wall. After 20 s a trial started with CS onset; 5 s later, the 1.6 mA scrambled shock was delivered through the grid-floor (unconditioned stimulus, US). Running to the opposite compartment within the 5 s CS-US interval precluded the foot-shock, immediately terminated the CS, and was scored as an avoidance response. Running to the opposite compartment after the US onset immediately cotermminated the CS and US and was scored as an escape response. Each daily training session consisted of 50 trials. The intertrial intervals (ITI) were of 15-25 s duration (mean = 20 s) and varied in a semi-random order. During intertrial intervals subjects were permitted to move in any direction, so they could cross away from or back into the compartment in which they had been previously. The next trial always started in the compartment where the subject was at the end of the ITI.

The latencies of instrumental responses were measured with an electronic timer, accurate to 0.1 s. Latencies of avoidance and escape responses were collected and analyzed separately. Each rat's median latency score for avoidance (or, separately, for escape) responses in a given daily session was used to calculate daily group means. Additionally, the median avoidance response latencies were calculated separately for trials 1-10 and 41-50 for each rat and session. For each rat the number of trials to the criteria of 3, 10, and 20 consecutive avoidance responses was calculated. For the purpose of data analysis a rat that failed to reach criterion was assigned a score equal to the number of its last escape trial. This practice, by assuming that the final series of avoidances would have continued until criterion was achieved, will tend to underestimate the actual number of trials that would have been required for the rat to reach criterion.

Group treatment

Before experiments rats were randomly assigned to three groups of 8 subjects each. During the nine daily training sessions, the groups differed only in the CS used. Group D was trained with darkness as the CS, Group N was trained with the onset of a 70 dB (re: 20 μN/m²) wide band (white) noise as the CS, and Group ND received both those stimuli given in compound. The darkness CS was provided by termination of the ceiling light in the compartment occupied by the rat. Just after a rat left the shock compartment it was again lighted; both compartments were illuminated during intertrial intervals. For Group N both compartments were continuously illuminated for the whole session.

The day after completion of the nine training sessions, the rats were used in additional tests in the same apparatus. All rats from Group ND were switched from the compound CS to the darkness CS during the 10th session. Then, during the 11th and 12th sessions they were again trained with the compound CS. For half of the rats from Group D and Group N during the 10th session, the compound CS of darkness plus 70 dB noise was used, while the remaining rats in these groups were given additional training with their original stimuli.

RESULTS

Avoidance performance

The groups differed markedly in the rapidity and effectiveness of avoidance learning. Rats of Group N and Group ND reached all criteria including also the criterion of 30 consecutive avoidances. Within Group D the criterion of 20 consecutive avoidances
TABLE I

The mean number of trials required by each group to attain a specified number of consecutive avoidance responses (not including criterion trials)

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<th>Criteria of consecutive avoidances</th>
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<td>3</td>
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<tr>
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<tr>
<td>Group N</td>
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<tr>
<td>Group DN</td>
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<td>Group D</td>
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was not reached by two rats. For rats of this group, data from an additional 50-trial test session were also taken into account. The mean number of trials to reach the criteria are presented in Table I. The ANOVA on the number of trials to the criteria of 3, 10, and 20 consecutive avoidances (not including the criterion trials) yielded effects of group \( (F_{2/21}=7.44, P<0.005) \) and criterion \( (F_{2/42}=35.13, P<0.001) \) and a significant interaction \( (F_{4/42}=5.00, P<0.005) \). Further Duncan tests for interaction showed that only the number of trials to 20 consecutive avoidances criterion in Group D differed from all other values.

**Escape latency**

The median daily response latency on shock trials for each individual rat varied from 5.23 to 6.45 s for the 1st training session and from 5.15 to 6.55 s for the 9th session. However, starting in the 5th session some rats trained with noise or compound stimuli emitted only avoidance responses. Thus, the stability of the escape response latency during two-way avoidance training was tested only for the 1st-4th sessions. A 3 (groups) x 4 (days) ANOVA of the daily median escape latencies showed neither group nor day effects and no interaction. It was found that in each individual rat of Group D the median escape response latency was 0.66 s. Nearly all rats from the other groups performed only avoidance responses during the last block of the 1st training session, making impossible similar comparisons for Group N and Group ND.

**Avoidance latency, between session changes**

In contrast to escape responses, the avoidance response latencies differed between groups and changed in the course of training. The overall group means of the individual medians for each session for rats trained to the noise (Group N), the complex (Group ND), and the darkness (Group D), were 1.92, 1.54, and 2.50 s, respectively. The 3 x 9 ANOVA of daily median avoidance latencies yielded no effect of group, but an effect of day \( (F_{8/168}=8.84, P<0.001) \) and an interaction \( (F_{16/168}=1.80, P<0.05) \). The shortest avoidance latencies were observed during the 1st (Group N and Group ND) or during the 2nd (Group D) training session. Further Duncan tests showed that in Group N avoidance latencies for the 4th, 5th, 7th and 9th sessions were longer than for the 1st session, in Group ND avoidance latencies for each consecutive session were longer than for the 1st session, and in Group D the avoidance latencies for the 6th session were longer than for the 2nd session.

The cumulative frequency distributions of avoidance and escape responses, emitted in consecutive 0.1 s intervals after the CS onset during the 1st, 2nd, and 9th sessions for Group N, are
Avoidance latency

Fig. 1. Cumulative frequency distribution of crossing response latencies in avoidance training with the noise warning signal (Group N) during the 1st, the 2nd, and the 9th training session.

As seen from the graphs, a minimum of 0.4 s was necessary to start the running response and cross the infrared beam located behind the central partition to terminate the trial. The distributions of avoidance response latencies suggested that the activating effects of the CS onset decay after 2 s (the 1st session) or 3 s (the 2nd session). The next impulse for the running response was given by the shock onset. At the end of training, on the 9th session, the cumulative distribution of response latencies had a different form. Responding was inhibited at the beginning of the CS-US interval. The number of avoidance responses for the 9th session surpassed the level of the 1st session only at 3.0 s and exceeded the level of the 2nd session at 3.5 s.

In contrast, no shift of the cumulative distributions to the right was observed in Group D (Fig. 2). At the beginning of training, during the 1st session, rats of this group showed a lower level of avoidance performance than the other rats. During the 2nd session the level of avoidance performance doubled in Group D. During the next sessions the performance of avoidance responses with latencies equal to or less than 2.0 s retained the initial position.

The data for Group ND were similar to those of Group N. For all groups the changes in the cumulative distributions of response latencies corresponded nicely to inference based on the Duncan tests from group means of individual median avoidance latencies. The overall mean avoidance latency lengthened during the training from 1.99 s at the 1st session to 2.68 s at the 9th session.

To evaluate the trend of changes in avoidance response latency in the course of training, the percentages of responses emitted with latencies equal to or less than 1.0 s, 2.0 s, 3.0 s, 4.0 s, and 5.0 s on consecutive sessions were compared within each group. For each time point the Spearman rho correlation coefficient between the group mean percentage of avoidance responses and the number of the corresponding training session was obtained. A positive rho value indicates an increasing, and a negative rho value a decreasing, trend of the percent-
Behaviors of avoidance responses at a given time point. The larger the absolute value of the rho coefficient, the fewer distortions from monotonicity were observed in the course of training. Results of such time trend analyses are presented in Fig. 3 for each group independently.

As is apparent in the graphs, the number of avoidance responses with latencies of 2.0 s or less decreased monotonically over the course of training for Groups N and ND. The tendency for the number of avoidance responses with latencies of 2.0 s or less to decrease in Group D was not statistically significant. In the second half of the CS-US interval the cumulative numbers of avoidance responses increased monotonically in all groups.

Avoidance latency, within session changes

In order to test within-session changes, median avoidance response latencies for trials 1-10 and 41-50 for each rat and session were compared. The avoidance response latencies were longer during the first block of trials than during the last block in a given session. Only during the 9th session small lengthening of avoidance latencies was observed in Group N and in Group D. Because some rats performed only escape responses in the first block of the early training sessions, the data were analyzed for the 3rd to the 9th sessions. A 3 (groups) x 7 (days) x 2 (blocks) ANOVA yielded an effect of blocks ($F_{1/21}=11.53, P<0.01$), no effect of the other factors, and no interaction. The differences in median avoidance response latencies for trials 1-10 and 41-50, averaged over sessions from 3 to 9, were 0.41, 0.29, and 0.24 s for Group N, Group ND, and Group D, respectively. Differences in median avoidance latencies between trials 1-10 and 41-50 were the greatest in the early sessions and were very small or even of a different direction at the end of training.

The effects of changing the CS

The sensitivity of the acquired temporal pattern of avoidance responding to changes of the warning signal was tested during additional sessions. In Group ND the test consisted of elimination of the salient component of the noise-darkness compound by presenting darkness alone. This change of the warning signal resulted in an immediate lowering of avoidance performance and rise in ITR rate. As seen in Fig. 4, this effect was seen from the very beginning of the 10th session. In consecutive 10-trial blocks the avoidance performance slowly recovered and at the fifth block reached the level observed during the previous sessions, when the compound CS was used. The marked increase in the shock density at the beginning of the 10th session resulted in an immediate and steadily increasing enhancement of the ITR rate. After reintroduction of
the compound CS in the 11th session, avoidance performance recovered immediately, whereas the previous low ITR rate was reached only toward the end of the 12th session.

The distribution of response latencies during the 9th session shown in Fig. 5 for Group ND is similar to that in Fig. 1 for Group N during the same session. The low level of responses at the beginning of the CS-US interval was preserved during the 10th session; however, the frequency of responses decreased in the late parts of the darkness CS presentation. The distribution of responses was markedly changed in the 11th session, when the darkness plus noise compound was reinstated. The cumulative distribution of responses shifted to the left, since most avoidance responses were emitted during the first 2 seconds of the CS-US interval. On the 12th session the frequency of responses at early phases of the CS presentation somewhat decreased, but was still much higher than before the use of the darkness CS. In fact, the distributions of response latencies at early phases of the CS-US interval during the 11th and the 12th sessions are similar to those observed in Group ND during the 1st and the 2nd training sessions.

Comparisons of cumulative response distributions of consecutive sessions from the 9th to the 12th day of training revealed significant differences from day to day (all $P'$s<0.001, Kolmogorov-Smirnov two-tailed test). The direction of change may be easily inferred from Fig. 5. The maximum vertical distance ($D_{\text{max}}$) between distributions for the 9th and the 10th sessions occurred at 4.3 s, between the 10th and the 11th sessions at 2.5 s, and between the 11th and the 12th sessions at the 1.9 s point. This indicates that the change from the compound CS to the darkness CS resulted in the decrease of avoid-
ance responses performed with long latencies, whereas reinstatement of the compound CS elicited enhancement of avoidance responses in early phases of the CS action.

The change from noise CS to compound CS in rats of Group N was without consequence for avoidance performance, distribution of response latencies, or ITR rate. On the contrary, the change from darkness CS to the compound CS in rats of the Group D resulted in changes similar to those observed in Group ND, when the compound CS was reinstated after one session of training with darkness CS. Avoidance responses with latencies of 1.0 s or less increased from 7.0 to 19.5, and with latencies of 2.0 s or less increased from 23.5 to 57.5 percentage of all responses. In this subgroup of rats the enhancement of avoidance responses emitted at the early portion of the CS-US interval was significant ($P<0.001$, Kolmogorov-Smirnov two-tailed test), and was not related to the increase in the ITR rate.

**DISCUSSION**

**Escape latency**

The results demonstrate the stability of escape response latency during two-way avoidance training. The shortening of escape latencies in the course of the 1st session in Group D, the slowest to learn avoidance, may be due to acquisition of a position habit or some other preparatory response mediated by the warning signal. In some rats escape crossing responses were performed 0.1-0.3 s after shock onset. In contrast, avoidance responses with latencies shorter than 0.4 s were not observed. Presumably, short-latency escapes occurred when the crossing response was initiated before shock onset and they may be considered "late avoidances" (Pacut and Tych 1982).

The concept of secondary learning processes (Bolles et al. 1976) cannot, however, be applied to explain the marked shortening of crossing response latency of rats trained to escape an unsignalled shock (Leeming et al. 1969). Shortening of escape response latencies was observed also when rats failed to avoid shock in a shuttle box (McAllister et al. 1979). Rats trained with stronger shock performed escape responses with longer latencies, but the shortening of escape latency was independent of shock intensity or the reward value of avoidance response. It was reported that the CS termination did not influence the escape latency (Kamin et al. 1959, Bixenstine and Barker 1964). The factors influencing variance of escape response latency are not known.

**Within session shortening of avoidance latencies**

Avoidance response latencies were shorter toward the end than at the beginning of nearly all training sessions. A similar reduction of two-way avoidance response latency within a training session was also observed earlier (Galvani and Twitty 1978, Bond 1981). In Starr and Mineka's (1977) experiments the mean avoidance response latency for the first three and the last three avoidances in a series of 27 consecutive non-shock trials shortened by half a second. In the McAllister et al. (1979) study significant shortening of avoidance response latency was observed during the 2nd session in rats trained with strong but not with weak shock. In dogs trained in a shuttle-box to avoid very strong shock the latencies of the jumping response shortened during 20 consecutive sessions, each containing 10 extinction trials (Solomon et al. 1953, Brush et al. 1955).

The within-session shortening of avoidance response latency occurred in parallel to the increase in avoidance performance and the rise in ITR rate demonstrated before (Zieliński et al. 1991). All these changes were pronounced during the early training sessions and, presumably, reflect behavioral activation elicited by high shock density at the start of each such session (Zieliński 1993).

**Hypothesis of inhibition of delay**

The extended avoidance training employed in the present experiment provided an opportunity to
discover long term changes, namely between-session lengthening of avoidance response latencies. The distinctiveness of this process depended on the CS modality. The effective reinforcement theory (McAllister et al. 1971, McAllister et al. 1976) seems to account for these findings better than traditional versions of the two-factor avoidance theory.

According to McAllister and some other theorists, the effective reinforcement for an avoidance response is positively related to the amount of fear reduction occurring with the response, and negatively related to the amount of fear present following the response. In our experiments rats trained with an auditory CS showed rapid avoidance learning and reached a high level of avoidance performance combined with very low ITR rate. On the contrary, when changes in illumination of the shuttle-box were used as a CS, rats learned avoidance slowly, reached a lower level of avoidance performance and exhibited a very high ITR rate. The present analysis showed that in the course of extended training the avoidance response latencies became longer, more so in rats trained with a noise CS than in rats trained with a darkness CS. More importantly, the probability of performing an avoidance response in the first half of the CS-US interval decreased in the course of training. This change, clearly apparent on cumulative frequency curves, may be interpreted as resulting from the development of Pavlovian inhibition of delay.

According to Pavlov (1927), the inhibition of delay results from a difference in proximity of the early and late portions of a CS to the unconditioned stimulus. The early phases of the CS, remote from the US, undergo a process of experimental extinction, whereas the late phases of the CS retain their excitatory properties. Thus, the changes may be considered to be the result of inhibition of delay only when at first both the early and late phases of the CS possess excitatory properties, and in the course of training the early phases of the CS partially or completely lose the excitatory properties, whereas the late phases retain them.

Exactly such changes were observed in the present experiment. The distributions of response latencies in Group N and Group ND indicate that more avoidance responses were performed in the first half of the CS-US interval during the early sessions than during the last sessions. The decrease in the frequency of early avoidance responses in Group D, to the extent that it occurred at all, was restricted only to the first second of the CS action.

Pronounced between-group differences in ITR rate indicate that less fear was conditioned to situational cues in Group N and in Group ND than in Group D (Zielinski et al. 1991). A hypothesis is proposed that conditions necessary for the occurrence of inhibition of delay in an aversive situation include a high level of fear to the CS terminated by an instrumental response and a low fear to situational cues.

**Fear of the CS and fear of situational cues**

The test sessions provide convincing arguments in favor of the hypothesis assuming that strong fear of the CS and low fear conditioned to situational cues are both required for the occurrence of inhibition of delay of avoidance responses. In Group ND the change from the salient compound CS to the less salient darkness CS resulted in an immediate enhancement of the ITR rate, denoting an increase in fear of situational cues. Nevertheless, the rats continued to emit a lowered number of avoidance responses in early portions of the CS-US interval. The marked increase of avoidance responses soon after the CS onset, and the distortion of inhibition of delay were observed only on the next session, when the reintroduced salient CS was acting on the background of enhanced fear of situational cues. Coexistence of strong fear of the salient CS and strong fear conditioned to situational cues is similar to conditions prevailing at the beginning of training, before formation of the inhibition of delay of avoidance responses. The similarity of the avoidance response distributions observed in the beginners and in overtrained but previously distressed rats may be explained by similar intensity relations.
between the two sources of fear in avoidance training.

In Group N the change from noise to compound CS was without any immediate consequence. The less salient darkness stimulus was fully overshadowed by the noise and did not elicit any enhancement of intertrial response rate. The inhibition of delay acquired during previous training was not disinhibited by this change in the warning signal quality.

In Group D the change from darkness to compound CS resulted in an immediate enhancement of avoidance responding in early portions of the CS-US interval. The new warning signal quality elicited a change in the distribution of avoidance response latencies without any increase in ITR rate. In fact, in this subgroup some further slow decrease of the ITR rate was observed. However, fear conditioned to situational cues had not been extinguished during previous training and the salient CS exerted its effect immediately. Thus, results of the test session indicate that some inhibition of delay of avoidance responses was in fact established in Group D in the course of the previous training.

In summary, the test applied to Group D follows the description of the experiments on delayed conditioned reflexes in Pavlov’s writings (1927). Our data support Pavlov’s statement that a new stimulus of moderate strength added to the previous one should exert a double effect on the conditioned response. The noise added to the darkness CS disinhibited responding in the early portion and inhibited responding in the late portion of the CS-US interval. Should the avoidance response be presented in the form of post-stimulus histograms with the CS onset as a reference point, the double effect would be easily visible. The test conducted in Group N was also in agreement with the statement that in some animals weak stimuli do not exert any effect on conditioned response rate. Thus, only the test in Group ND, which was designed differently, seems to indicate the necessity of another approach to explain the long-term changes in avoidance response latencies.

We believe that the influence of warning signal modality on the temporal pattern of avoidance response performance is related to differences in signalling properties of the warning stimulus termination. An insufficient discrimination of the opposite changes in illumination level was thought to be responsible for high fear conditioned to situational cues during avoidance training with visual CS (Zielinski et al. 1991). On the contrary, extended training with a salient auditory CS provide conditions for the development of inhibition of delay in two-way avoidance training: a high level of fear elicited by a warning signal and low fear conditioned to situational cues.

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