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How do rats cope with the two-way escape problem in a homogenous shuttle box?

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Abstract. The behavior of 25 rats trained in a homogeneous shuttle box to escape unsignalled grid-shock was analyzed. Three categories of escape were distinguished: (1) species-specific fly away from the charged grid, (2) long-latency crossing preceded and accompanied by other behaviors that compete with the escape response, and (3) short-latency escape which followed an anticipatory postural pose. The animals displayed species-specific fly away only during the initial trials of a session. Subsequently long-latency crossings develops, reflecting a resistance to enter the opposite compartment. A measure based on a comparison of escape latency distributions in the two halves of the 1st session discriminates between good and poor learners. Subgroups of good and poor learners differed in performance efficiency in all five training sessions. Good learners were able to overcome the resistance to enter the opposite compartment and recall the learned short-latency escape.

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INTRODUCTION

Contingency between stimuli used and the required response determine rapidity of learning and stability od the acquired response. The opportunity to prevent shock application with instrumental avoidance response results in more stable performance than the regular pairings of the conditioned stimulus and shock used in classical defensive conditioning (Brogden et al. 1938). It was recognized also that introduction of unsignalled shock trials terminated with the instrumental escape response have deteriorating effect on the trained avoidance response (Mowrer 1940) even in case when the morphologes of shock-avoidance and shock-escape responses were alike (Mowrer and Lamoreaux 1946).

Comparison of two-way and one-way active avoidance learning in conventional shuttle boxes provides another example for the role of the experimental procedure. A trial starts with presentation of a warning signal (conditioned stimulus, CS), such as opening of the guillotine door between compartments or termination of the overhead light in the occupied compartment. After a predetermined period of time a grid shock is added to the CS. To avoid painful stimulation or to terminate its action, the subject is required to leave the shock compartment and enter the opposite compartment. In the two-way procedure the next trial starts after an intertrial interval (ITI), with presentation of the CS and-or shock in the compartment occupied by the subject. Therefore no handling is involved. The next crossing response consists of reentering the previously shocked compartment. In contrast, in the one-way procedure the subject is removed by the experimenter from the safe compartment at the end of a trial and after the ITI is put back again into the same previously shocked compartment. Usually the shocked and safe compartments in the one-way apparatus are distinctive and easily discriminated from each other.

Experimental data shows that a criterion of ten consecutive avoidance responses was reached in the one-way procedure after 3-5 trials, whereas in the two-way procedure the same criterion required several dozens of training trials (Theios and Dunaway 1964). A strong tendency to not return to the previously shocked compartment has been noted (Theios et al. 1966). A rat trained under the two-way procedure may freeze or, instead, vigorously run and jump when on the charged grid but does not readily enter the opposite compartment of the box. This behavior which effectively interferes with the initiation of the escape response was termed a "staying response" (Theios et al. 1966). The staying responses were enhanced with shocks of higher intensity, which resulted in slowing down two-way avoidance acquisition (Levine 1966, McAllister et al. 1971). Any condition which enhances the rat's resistance to return to the previous shock compartment impairs two-way avoidance acquisition (for review see: Bignami et al. 1985). In contrast, the one-way escape response was acquired more readily with intense than with mild grid shock (Franchina 1969, Dieter 1976). Introduction of safe platforms turns the shuttle box into a one-way experimental situation (Modaresi 1975).

An intriguing question is how rats cope with conflicting tendencies to flee from the charged grid vs. resisting a return to a previously dangerous compartment. We know relatively little how rats behave in the two-way escape procedure. Much of what we know comes from the early acquisition trials of signalled avoidance training. Brush (1966) showed that rats trained in two-way avoidance emit escape responses with increasing latencies reaching a peak around the 6th trial and then decreasing. The same pattern of changes in escape latency was noted in both animals that learned to avoid and in those that did not, but learners started faster, rose to a lower peak and decreased to a faster asymptote than non-learners. Unfortunately, there was no follow up of this early report. Only rarely has it been noted that the mechanisms of response evocation and control in avoidance and in escape procedures differ markedly from one another (Ehrman and Overmier 1976). We suggest that the variability of strategies employed by individual rats has to be examined first in an unsignalled escape situation. The use of a warning signal which rapidly acquires fear-evoking properties only introduces additional complexity.

In contrast to the work of Bolles (Bolles 1970, 1978, Bolles and Collier 1976, Bolles et al. 1976) which suggest an invariance of latency to escape from shock, we assumed that the fly away from a charged grid - the species-specific defensive reaction (Bolles 1970) - might be displayed only during the initial trials. Then, a lengthening of the latency of the crossing response should be observed, denoting the presence of a conflict situation. This should be accompanied by changes in the frequency and temporal distribution of various modes of behavior displayed by a rat. Finally, some subjects may acquire a definite preparatory postural response which would allow rapid passage from the actual shock to the opposite compartment.

To document the nature of the behavioral expression of the conflict each rat in this experiment was observed during each training session, its discrete reactions coded and noted sequentially. Attempts to differentiate between good and poor learners resulted in a division of the animals into three subgroups marked by different interrelations of behaviors during the first and subsequent sessions.

METHODS

Subjects

In these experiments, the rules established by the Ethical Committee on Animal Research of the Nencki Institute and based on disposition of the President of Polish Republic were strictly followed.

The experiment was conducted on 25 adult male Möll-Wistar rats bred in the Nencki Institute, experimentally naive, and weighing 320-360 g. Subjects were kept in groups of five in home-cages (43 cm long, 25 cm wide, 18.5 cm high), containing food and water ad libitum. A natural light-dark cycle from external illumination was maintained. Rats were trained in the morning or early afternoon and experiments were completed within 50 days of May and June.

Apparatus

The shuttle box apparatus was 62 cm long, 18 cm wide, and 29 cm high with walls of opaque white acrylic. The box was divided in half by a wall with a rectangular (7 cm wide, 10 cm high) opening situated at the level of the grid floor which permitted passage from one side of the shuttle-box to the other. Each compartment was covered with a movable transparent acrylic ceiling and illuminated by a 5 W lamp mounted centrally just below the ceiling. The response of crossing through the opening was detected 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 rods, 0.4 cm in diameter, that were parallel to the central partition 1.5 cm apart from each other. The shuttle-box apparatus was placed in a sound-proof dimly lighted room. TV setup permitted direct viewing and recording the subjects' behavior in an adjoining room, where equipment for automatic programming of the experiment and recording of data was located.

Procedure

The rats were assigned randomly to groups of five subjects each. Each animal was habituated to the situational cues of the apparatus for 10 min on two consecutive days. Training started on the next day. At the beginning of each session, the rat was placed in the left compartment of the shuttle-box, close to and facing the end wall. The first trial started 20 s later. Each session consisted of 50 trials. The intertrial intervals (ITIs) lasted 14, 20, or 26 s (mean = 20 s) and varied in a mixed order. During the ITIs, the animals 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 located at the end of the ITI.

In the unsignalled escape procedure rats were trained to escape from shock given without any other change in the experimental situation. Therefore, no CSs or movable door between the two compartments of the shuttle box were used in our experiments. Similarly, a variable ITI was used to prevent the possibility of temporal conditioning. The nominal 1.6-mA scrambled, pulsed DC shock (50 Hz pulse rate) delivered through the grid floor could be escaped by running to the other compartment or was terminated automatically when 30 s had elapsed.

Rats were trained for either 1, 3, or 5 sessions. Thus, data were obtained from all 25 subjects in the first session, from 20 subjects in the second and third sessions and from 15 subjects in the fourth and fifth sessions. In daily sessions five subjects were trained consecutively in the same order and about the same time. After completing the scheduled number of sessions another squad of rats started training. The order of squads submitted to different amounts of training was determined beforehand by chance.

Measures

The basic measure of behavior was latency of the crossing response. All timed events during training trials, response latencies and ITRs were controlled by electronic timers (± 0.01 s) and control circuits. Efficiency of behavior was indexed by the shock duration, i.e., escape latency on a trial or averaged over a session. The difference between total shock duration on trials 1-25 and 26-50 was used to index learning within a session. Median latencies together with the semi-interquartile range, i.e. Q = (Q75%-Q25%)/2, provided estimates of variability of the escape latencies. A 5-trial running average (Run. Aver. Esc. Trial$_n$ = (a_{n-4} + a_{n-3} + a_{n-2} + a_{n-1} + a_n)/5) of escape latencies was used to smooth response latencies as a function of trials within a session.

The experimenter (A.S.) observed and sequentially coded the behavior of each rat throughout training. Direct observations "on line" from the monitor were confirmed subsequently, if necessary, from video tapes of each session. Overt behavior was discriminated and coded in the 18 categories:

(1) direct escape response - running to the opposite compartment immediately after the shock onset;

(2) staying response - delay of the escape response, observed as freezing on a charged grid or any other behavior interfering with the escape response;

(3) intertrial response (ITR) - running to the opposite compartment during ITI;

(4) orienting - turning of a body and/or orienting of a head towards the doorway and maintaining this position for at least 1 s;

(5) preparatory response - orienting maintained up to subsequent shock application;

(6) head-toss response - at least two movements of the head in succession from the opening and backwards;

(7) freezing;
(8) jumping;
(9) jumping up;
(10) vocalization;
(11) biting;
(12) tail up;
(13) chatter;
(14) defecation;
(15) walking;
(16) rearing;
(17) smelling;
(18) grooming.

Freezing and orienting behaviors were recorded only after counting of at least two ticks of a 2-Hz metronome. After completion of this study, the reliability of reaction coding was tested. An additional group of 10 rats was trained for one session, observed by the same (A.S.) experimenter and recorded on the videotape in parallel. Two weeks later the behavior of rats was again coded but now from the videotape records. Coding of discrete reactions done on-line and from videotape recordings showed high accordance similar to behavior coding in an appetitive situation (Stokes 1995). Code agreement was calculated by dividing total agreements between the number of reactions of each category in both coding procedures by total agreements added to total disagreements and for individual categories of behavior was: 0.95 ± 0.03 for direct escape, 0.88 ± 0.07 for staying responses, 0.86 ± 0.14 for freezing, 0.91 ± 0.09 for preparatory responses, 0.91 ± 0.16 for biting, and 0.84 ± 0.04 for grooming.

RESULTS

Within-subject variability of escape latency

Substantial variability of escape latency has been observed during the course of the first session. Figure 1 illustrates typical data for two rats. After placement in the shuttle box Rat 188 exhibited exploratory activity with two crossings. The first shock elicited a rapid escape re-

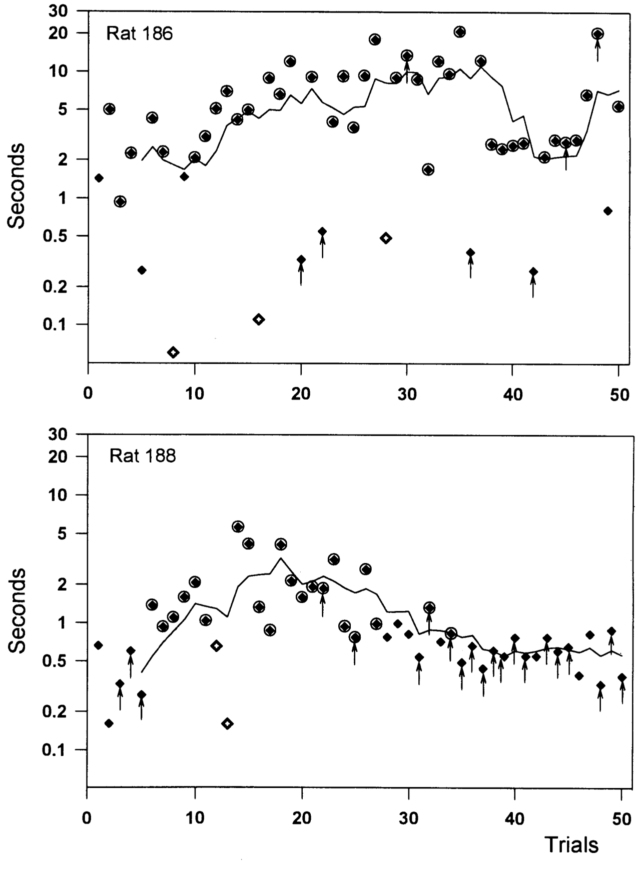

Fig. 1. Changes in escape responses latency on consecutive trials of the 1st session for a typical poor (Rat 186) and a typical good learner (Rat 188). Diamonds denote latency of an escape response, diamonds in a circle denote "staying" response, diamonds with + inside denote an artifact, arrows below diamonds denote postural preparatory response. The line plots the running average of the preceding five escape latencies (see text for details).

sponse unaccompanied by vocalization. During the ITI the rat displayed head tossing, exploratory sniffing followed by crossing and sniffing the grid in the previously shocked compartment. The next shock occurred when the rat was facing the rear of the box. The rat backed through the doorway after which it froze for 10 of the 26 s ITI whereupon head tossing and sniffing/exploration followed. This was stopped by the third shock which elicited a rapid escape followed by sniffing the doorway, crossing and sniffing the grid followed by turning toward the doorway and freezing. By the 6th trial the rapid escape disappeared and all shocks elicited audible vocalization. Orientation toward the doorway disappeared and head toss and postural movements were brief. Shock elicited freezing or very active jumping toward the ceiling and across the shock compartment all of which produced long escape latencies. The short latencies on trials 12 and 13 are an artifact of tail flexion which triggered the photocells to terminate the shock. Typically, the rat oriented toward the opening at the beginning of ITI and then froze until the next trial. Around the 30th trial there was a transition period in which freezing followed by staying responses gave way to preparatory postural responses followed by direct escape responses, and short latencies.

In summary, this rat (188) displayed three stages of learning: (1) a brief period of exploratory locomotory and sniffing behavior with short-latency escape flight responses during shock, (2) freezing during the ITIs and long latency staying responses during the shock, (3) reappearance of preparatory postural responses and direct, short-latency escape responses.

Similar stages were observed in rat 186 with important differences. Already the second shock elicited jumping across the compartment followed by arrest in front of the doorway, vocalization, and escape after several seconds of freezing. The first preparatory response, which consisted of orienting toward the doorway, was noted at the beginning of the ITI following the trial 19. The next shock elicited a rapid escape, but on remaining trials of the session only a few short-latency escapes were interjected in a long series of long-latency staying responses. Rat 186 never got to the orientation/preparatory movement stage and just froze during the ITI.

Segregation of "good" and "poor" learners

We examined a number of measures from the first session in an attempt to identify subsequent good and poor learners, but found them to be too variable to be useful predictors. For example, number of initial fleeing responses (Mdn = 3, range 0-15), staying responses (Mdn = 17, range 2-44), and direct escape (Mdn = 32, range 5-46), were not able to segregate good and poor learners. Similarly, neither the difference in of staying and direct escape responses, as tested by the binominal test (Siegel 1956), nor an index based on the sequential performance of direct and staying responses, as tested by the runs test (Siegel 1956) possessed any predictive power. Therefore we turned to time parameters and found that the difference in total duration of shock received during the first and second halves of the first session was a good predictor.

Table I presents the mean (± SE) and median (± semi-interquartile range) escape latencies for Rats 186 and 188 and by the group of 25 rats during the first (I) and the second (II) halves of the 1st session of unsignalled escape training. Comparison of the means and medians indicates that the distributions are strongly positively skewed.

Cumulative frequency distributions of escape latency, using 0.5-s bins, were constructed for each half of the

TABLE I

Temporal indices of escape performance during the first (I) and the second (II) halves of the first session for Rat 186, Rat 188 and for all 25 subjects trained in unsignalled escape. In consecutive lines: (1) mean escape latency and standard deviation for halves of the session, (2) median escape latency and semi-interquartile range. All indices are given in seconds

	Rat 186		Rat 188		All rats	
Half of session:	I	II	I	II	I	II
Mean latency	4.30 ± 3.24	7.21 ± 6.51	1.58 ± 1.38	0.76 ± 0.45	2.86 ± 2.78	2.61 ± 2.54
Median latency	4.01 ± 2.05	4.18 ± 3.87	1.10 ± 0.63	0.66 ± 0.13	1.76 ± 1.35	1.80 ± 1.08

first session, and the difference (S_1-S_2) for each 0.5-s bin was calculated. A positive value of the difference indicates shortening of escape latency (improvement of escape performance) whereas a negative value denotes lengthening of escape latency (impairment of performance) within a session. The Kolmogorov-Smirnov two-sample test was used to compare the two distributions for each animal. The statistic, which allows an inference about agreement or disagreement of two sets of sample values was the maximum vertical distance (D_{max}) between two cumulative distributions (Siegel 1956). The D_{max} values for Rats 186 and 188, whose behaviors during the first session were described previously, are shown in Fig. 2. For samples $n_1 = n_2 = 25$ the D_{max} 10 is significant at $P<0.05$. As seen from the figure, for Rat 188 the positive value of D_{max} was due to a shift of the distribution to the left and denotes a shortening of escape latencies in the second half of the session. In contrast, for Rat 186 the D_{max} was negative, denoting a lengthening of escape latencies in the second half of the session. This change was not significant and the point of D_{max} was located within long-latency staying responses.

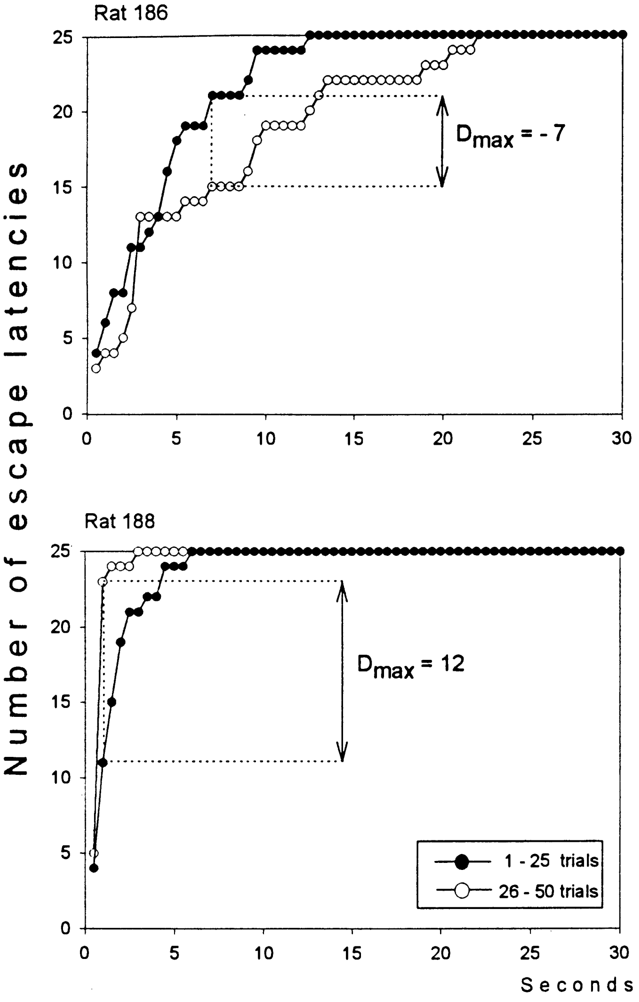

Fig. 2. Cumulative frequency distributions of escape response latencies performed during the first (S_1) and the second (S_2) half of the 1st training session for rats typical of poor (Rat 186) and good (Rat 188) learners. For Rat 186 the D_{max} was negative and located at 7.0 s, whereas for Rat 188 the D_{max} was positive and located at 0.5 s.

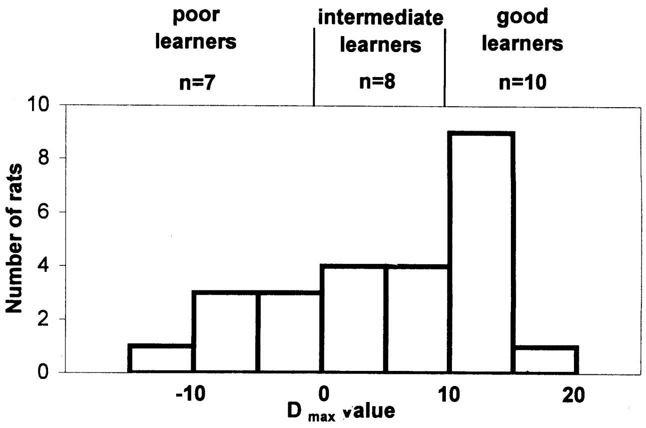

Fig. 3. Histogram of values of the maximum discrepancy (D_{max}) between cumulative distributions of escape response latencies emitted during the first (S_1) and the second (S_2) half of the 1st training session for all 25 rats trained in unsignalled escape.

The results of the Kolmogorov-Smirnov two-tailed test for all 25 rats are presented in Fig. 3. A significant improvement of escape performance was found in ten rats. For all of these rats the D_{max} between the compared distributions was located within the shortest escape response latencies, i.e., no more than 1 s. In contrast, significant impairment was observed only in one rat with D_{max} located at the 3.5 s point which was maintained for latencies up to 8.5 s. In all but one rat having negative differences the D_{max} between the cumulative distributions was located within long-latency responses (mean = 4 s, range 0.5-9.0 s). There was a positive correlation between the D_{max} value and the difference of shock duration (Sh_I-Sh_{II}) received during two halves of the session, $r = 0.72$, $n = 25$ ($P<0.001$).

These data were used to segregate the rats into three subgroups: good learners (those with $D_{max}>10$, $n = 10$), poor learners (those with $D_{max} <0$ value, $n = 7$), and rats with intermediate learning (with $0< D_{max} <10$, $n = 8$).

Interrelations of behaviors during the 1st session

Figure 4 presents changes in the frequency of responses, in blocks of five trials during the first session, for subgroups of poor (*n* = 7), intermediate (*n* = 8) and good (*n* = 10) learners based on the preceding analysis of cumulative escape latencies. Panels A and B reflect the behavior while they are being shocked, whereas panels C and D present reactions during ITIs. Latencies of escape responses (Fig. 4A) lengthened up to the 15th trial and then the subgroups diverged. A 3 x 10 (subgroup x blocks) mixed-design ANOVA yielded significant effects of block $F_{9,198}$ ($P<0.001$) and interaction $F_{18,198} = 2.34$ ($P<0.002$). Planned comparisons revealed curvilinear changes of response latencies within each subgroup. For good learners the response latencies of Block 1 were shorter than in Block 3, which in turn were longer than in each of Blocks 5-10. For intermediate learners the response latencies of Block 1 were shorter than in Block 4, which in turn were longer than in Block 9. For poor learners response latencies of Blocks 1 and 2 were shorter than in each of Blocks 4-8, whereas latencies of Blocks 6 and 7 were longer than in each of Blocks 8-10. Further post-hoc Neuman-Keuls tests showed that response latencies for good and poor learners differed in Blocks 4-10 except for Block 5. Similarly, intermediate and poor learners differed in each of Blocks 6-8.

As seen in Fig. 4B, the differences in learning efficiency were related to changes in the proportion of staying responses performed during the session. Significant effects of block and interaction were similarly revealed by 3 x 10 ANOVA: $F_{9,198} = 8.58$ ($P<0.001$) and $F_{18,198} = 4.48$ ($P<0.001$), respectively. Planned comparisons revealed curvilinear changes in the percentage of trials with staying responses for intermediate and good learners. In good learners the percentage of trials with staying responses was higher in Blocks 2-4 than either in Block 1 or in Blocks 6-10. Similarly, in intermediate learners the percentage of trials with staying responses increased from Block 1 to each of Blocks 3-4 and decreased from each of Blocks 3-5 to Block 6 and to Block

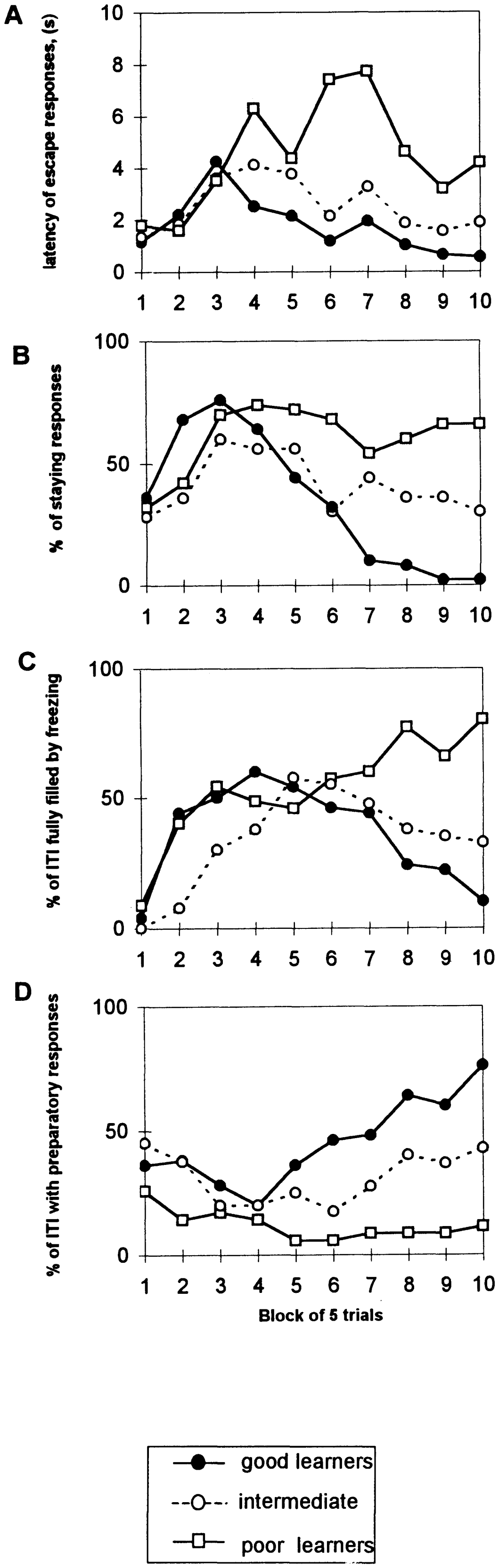

Fig. 4. Percentages of trials or intertrial intervals in which certain behaviors were observed in consecutive 5-trials blocks of the 1st session in subgroups of poor, intermediate, and good learners. A, mean latency of shock-terminating responses. B, percent of staying responses. C, percent of intertrial intervals fully occupied by motionless freezing. D, percent of intertrial intervals with postural preparatory responses maintained up to the onset of the next trial.

10. In poor learners the percentage of trials with staying responses significantly increased from the 1st to the 3rd Block and was maintained on a high level until the end of the session. Further post-hoc Neuman-Keuls tests showed that the percentages of staying responses for good and poor learners differed in each of Blocks 5-10.

Spearman rank correlation between the mean latencies of escape responses and the number of staying responses in each block of 5 trials revealed a positive correlation for each subgroup of rats ($r_s = 0.83$, $r_s = 0.88$, $r_s = 0.67$ for good, intermediate, and poor learners, respectively, P's<0.001).

Figure 4C presents changes in the number of ITIs occupied fully by freezing behavior. Instances of ITIs with uninterrupted freezing were very rare during the first block of 5 trials, which then rose rapidly reaching about the 50% level in the middle of the session. Subsequently the curves diverged: poor learners showed a further increase in the number of ITRs with freezing behavior, whereas in good learners instances of freezing decreased reaching a low level toward the end of the session. Intermediate learners slowly increased their freezing to the middle of the session and then slowly decreased their freezing toward the end of the session. A 3 x 10 (subgroup x blocks) mixed-design ANOVA yielded significant effects of block $F_{9,198} = 8.25$ ($P<0.001$) and interaction $F_{18,198} = 3.23$ ($P<0.001$). Planned comparisons showed that poor learners increased ITIs with freezing from Block 1 to Block 2 and then from each of Blocks 2, 4, and 5 to Blocks 8 and 10. For intermediate learners an increase was observed from Blocks 1 and 2 to each of Blocks 3-10. For good learners the initial increase of ITIs with freezing from Block 1 to each of Blocks 2-7 changed to a significant decrease from Block 7 to Blocks 9 and 10. Post-hoc Newman-Keuls tests revealed that good and poor learners differed at Block 8 and 10, whereas intermediate and poor learners differed only at Block 10.

A low level of freezing makes it possible for other behavior to occur. As seen from Fig. 4D, postural preparatory responses were observed as early as Block 1 when most of the trials were terminated with fleeing responses. Then, postural preparatory responses, and also sniffing and rearing reactions decreased. Postural preparatory responses in poor learners disappeared nearly completely during the second half of the session. In contrast, in good learners an increase in the frequency of postural preparatory responses occurred, which, toward the end of the session, preceded most of the shocks. The late preparatory responses were accompanied by head toss: rapid movements of the head toward the doorway and then toward a rear wall.

A 3 x 10 mixed-design ANOVA of the frequency of preparatory responses yielded significant effects of subgroups $F_{2,22} = 4.96$ ($P<0.02$) and blocks $F_{9,198} = 2.76$ ($P<0.005$). Planned comparisons for good learners yielded differences between each of Blocks 1-5 and each of Blocks 8-10, and also between each of Blocks 6-7 and Block 10. For intermediate learners curvilinear changes were revealed, i.e., the number of preparatory responses decreased from Block 1 to Blocks 3, 4 and 6 and then increased from Block 6 to Block 10. Post hoc Neuman-Keuls test showed that the number of preparatory responses by good and poor learners differed in each of Blocks 8-10.

All rats dramatically decreased the number of crossings immediately after the first shock presentation. Thus, the mean rate of spontaneous crossings (ITRs) before the first trial was 3.24 per min, whereas for the remainder of the 1st session the rate was only 0.42 ITRs per min. Some recovery of the ITR rate during the 1st session was observed in good learners. Similarly, grooming behavior was strongly depressed after the first session of training. Nearly 80% of the grooming responses were observed immediately after an escape response and another 6% were seen just after an ITR. Furthermore, most of the grooming occurred during the second half and never at the beginning of the training sessions.

Escape responding after prolonged training

The within- and between-subject variability of escape performance make it difficult to characterize the course of two-way escape learning. Thus, in Fig. 5 three functions are presented: for good, intermediate, and poor learners as segregated according to the results of the first session. Only data for the 15 rats which completed 5 sessions training are presented and analyzed.

In the course of training a decrease in the mean escape latency occurs in all three subgroups. A 3 x 5 (subgroup x sessions) mixed-design ANOVA yielded a significant effect of session, $F_{4,480} = 6.72$, $P<0.001$. Post-hoc Newman-Keuls test revealed a significant difference between the good and poor subgroups in the 1st session ($P<0.05$). Planned within-subgroup comparisons revealed a significant shortening of latencies in intermediate learners from the 1st to the 2nd session ($P<0.01$) and in poor learners from the 1st to the 3rd session ($P<0.05$). The posi-

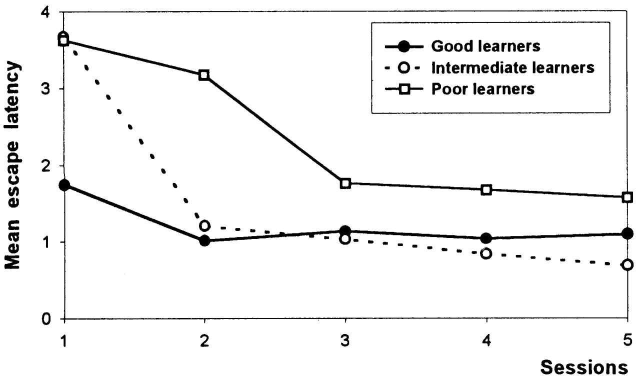

Fig. 5. Mean escape latency for subgroups of poor, intermediate, and good learners during the training sessions.

tive correlation between the D_{max} value and the difference in shock duration received during the first and second halves of a session (Sh_I-Sh_{II}) was found in all five sessions, P's<0.01, 0.01, 0.10, 0.05, 0.05, respectively (Pearson r correlation).

For the 15 rats which completed five sessions of training the frequency of direct escape responses increased from the first session to reach a plateau of 80% by the 3rd session. Four of the 15 subjects came to perform exclusively direct escape during the entire 50-trial session, but only one rat did this consistently on three consecutive days. Median escape latency during a session in which a given rat performed only direct escape responses was close to 0.4 s.

Any shortening of escape latency within a session (high D_{max} value) was a consequence of a decrease in the frequency of staying responses. For the 1st session the correlation coefficient between the difference in proportion of staying response (St_I-St_{II}) and the difference of shock duration (Sh_I-Sh_{II}) for the first and second halves of a session was $r = .54$, $n = 25$ ($P<0.001$). A similar analysis for the 3rd session with $n = 20$ subjects, also yielded a positive r which, however, did not differ significantly from zero, presumably due to an overall decrease in the number of staying responses in the course of training. The decrease of staying escape responses over sessions 1-3 was confirmed by a repeated measures ANOVA on 20 subjects which completed 3 sessions of training ($F_{2,38} = 17.67$, $P<0.001$). Moreover, the number of intertrial intervals filled with freezing behavior decreased and the shape of the distribution of this index changed dramatically from the 1st (Mdn = 21; Mean = 21.0) to the 3rd (Mdn = 7; Mean = 15.6) session. Thus, the Wilcoxon matched pairs test was used, which indicated that the difference is significant at $P<0.02$. The number of ITIs in which postural preparatory responses occurred immediately before the onset of the next shock increased from the 1st to the 3rd session ($P<0.001$, t-test).

The process of consolidation of the direct escape responses was traced in 20 subjects which completed three sessions of training. An analysis was done independently for three subgroups of rats distinguished on the basis of the D_{max} value between the cumulative distributions of response latencies for the two halves of the 1st session. Sequences of consecutive appearance of either direct escape or staying responses were examined. Starting on the second trial, sequences of direct-direct (Dir-Dir), staying-staying (St-St), staying-direct (St-Dir), direct-staying (Dir-St) pairs of responses were noted, summed across subjects for each trial and calculated as percentages. Within each panel of Fig. 6 the running averages of these statistics are presented.

As can be seen in the Figure, the three subgroups of rats differed markedly not only in the proportion of direct and staying responses but also in the dynamics of occurrence and decay of the different categories of shock-terminating responses. At the very beginning of the 1st session (left panels) the Dir-Dir sequence predominated in all group of rats. Good learners (n = 8) were marked by a rapid growth and subsequent rapid decay of the St-St sequence. Starting from the middle of the 1st session the Dir-Dir sequence predominated, reaching nearly 90% towards the end of the session. This prevalence of Dir-Dir sequence denotes a solution of the problem. Good learners solved the problem early with a consistent decrease in the number of ITIs occupied with freezing. The further increase in the frequency of the Dir-Dir sequence was correlated with a rise of ITI with preparatory responses (Fig. 4C and D). In the other subgroups the early growth of the St-St sequence was not followed by its subsequent decay. In the subgroup of intermediate learners (n = 6) the St-St sequence was only slightly less frequent than the Dir-Dir sequence. Poor learners (n = 6) showed a rapid decrease of the Dir-Dir sequence and a continued predominance of the St-St sequence during most of the 1st session. Changes in the categories of shock-terminating responses (Dir-St and St-Dir sequences) were rather infrequent in any of the subgroups.

As seen from the middle panels, during the 2nd session the Dir-Dir frequency increased and the St-St sequence decreased in all subgroups of rats, but the ordering of relations between good, intermediate and poor learners were fully maintained. During the 2nd ses-

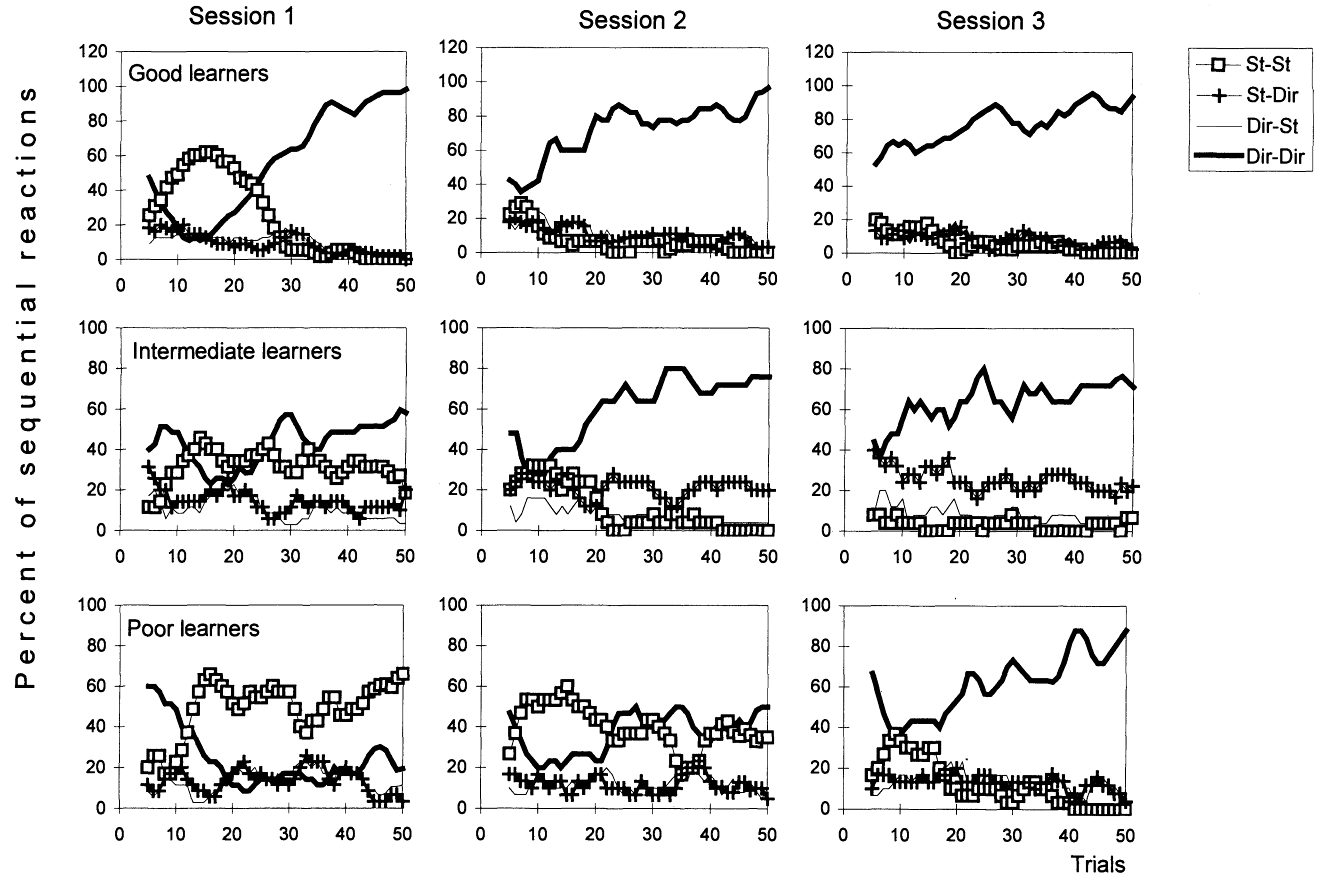

Fig. 6. Sequential patterning of staying and direct escape responses for good (the upper row), intermediate (the middle row), and poor (the bottom row) learners during the 1st (the left panels), the 2nd (the middle panels), and the 3rd (the right panels) sessions.

sion the initial rise of the St-St sequence in all groups was even more rapid than during the 1st session. However, good learners were able to suppress their staying responses very rapidly. Intermediate learners also inhibited the staying responses but after some delay. Poor learners did not suppress their staying responses even by the end of the 2nd session.

During the 3rd session (the right panels) the Dir-Dir frequency predominated in all groups. However, the subgroups differ in the percentages of this sequence. Intermediate learners were marked with relatively high frequency of the Dir-St sequence, and poor learners still performed more St-St sequences than the other subgroups. Nevertheless the differences among the three subgroups dissipated in the second and the third sessions. The data presented here indicate that learning cumulated over the course of training and most of the rats improved their efficacy in escaping shock. However, there was little retention from the final trials of one session to the beginning of the next session. In most rats there is no evidence of monotonicity in learning this unsignalled escape task.

DISCUSSION

The present study provides basic information about the sequence of changes in behavior that occur during learning to escape grid-shock in the two-way shuttle box. In contrast to avoidance learning, which has been studied extensively, unsignalled two-way escape learning has not been systematically studied. This paper documents the nature of the behavioral expression of the conflict inherent in the two-way procedure.

A naive rat, previously habituated to the apparatus cues, when confronted for the first time with pain from a charged grid immediately arrests its investigatory behavior and responds by rapidly fleeing from the shocked grid. On subsequent trials shock is presented in both compartments, and the whole experimental apparatus becomes dangerous and elicits other species-specific defensive reaction, e.g., freezing. After about 5 trials or so, the rats freeze throughout approximately 50% of the ITIs. The shock of the next trial, however, disrupts this freezing and instead of locomotion toward the doorway, the rat usually moves backward or exhibits frantic running and jumping around the compartment on the charged grid. All of these reactions in the shocked compartment are accompanied by audible vocalization. The initial stereotyped escape response changes to a variety of behaviors reflecting the resistance of a rat to enter a compartment in which it had been shocked. Theios et al. (1966) proposed labeling this behavior a "staying response". This staying response developed not only in poor but also in good learners.

One of the main conclusions of the present study is the need to distinguish among three categories of escape responses elicited by grid shock in the shuttle-box: a species-specific fly away reaction, a long-latency staying response and a short-latency direct escape preceded by a postural pose. Changes of the emotional level were related with the occurrence of one or another class of escape response. Thus, contrary to the original notion about invariance of response latency in a shuttle box (Bolles et al. 1970), both avoidance response latency (Zieliński et al. 1995) and escape response latency (this study) do vary in a systematic way.

A crucial question concerning unsignalled two-way escape learning is to determine the mechanisms responsible for overcoming the resistance to enter the opposite compartment. As mentioned by Bolles (1978) the fear-reduction hypothesis is not able to provide a resolution of this question, because the shock-terminating escape response constitutes a run from a new fear to the old one; the overall reduction in fear level is small, so the amount of reinforcement is also small. However, it has been shown that not only termination of shock, but also the shortening of shock duration reinforces the crossing responses elicited by pain (Kamin et al. 1959). Our data support this notion. Good learners shortened their escape latencies before they reliably decreased their staying responses (Fig. 4A and B).

It has been postulated that in the experiments in which shocks are repetitively and regularly applied, the aversiveness of the situation is reduced (Azrin 1956), and the lowest aversiveness of the conditioned temporal stimuli would be expected soon after shock termination (Anger 1963). In the shuttle situation all places are aversive (or are becoming so) and only discriminating between the dangerous time, when the shock comes on, and the safe time, soon after shock termination, is possible. The time discrimination hypothesis has been recently supported by data indicating anticipation of the next trial by rats trained in two-way avoidance. It has been shown that termination of the CS by an avoidance response resulted in a sharp reduction of the ITR rate denoting a decrease in the excitation level of the rats. Then as the ITI progressed, the ITR rate gradually increased to a maximum at or near the end of the ITI, i.e., in anticipation of the onset of the next trial (Zieliński and Nikolaev 1997).

The results of this experiment suggest that in the course of unsignalled two-way escape training rats learned a time discrimination and changed their behavior in accordance with the temporal oscillation of dangerous and safe periods. Expectation of the next shock was evidenced by the response of orienting toward the doorway followed by tonic postural preparatory responses. Good learners learned these preparatory responses before they showed a reliable decrease in the number of ITIs in which they froze throughout the interval (Figs. 4C and D). Having learned these preparatory responses, good learners then exhibited short-latency escape responses at shock onset. Poor learners required 2-3 sessions before reaching analogous level of performance.

An increase in escape latency during the early trials of avoidance training reported by Brush (1966) was interpreted as resulting from an increase in freezing interfering with the escape response. We suspect that the subsequent decrease in escape latency in Brush's study was due to learning a time discrimination which enabled anticipation of the next shock presented at a fixed time interval after the previous one.

Brush's study emphasized individual differences in the defensive behavior of rats. It is now well established that genetic factors are responsible for differences in emotional reactivity and aversive learning ability of selected strains of rats (Broadhurst and Bignami 1965, Gendron and Brush 1966, von Kluge and Brush 1992, Zhukov and Vinogradova 1994). Numerous experiments employing a number of different tasks showed that rats selectively bred for good shuttle box avoidance learning were less emotionally responsive than rats selectively bred for poor shuttle box avoidance learning, whereas neither strain differed in absolute sensitivity to electric shock (Brush et al. 1985, 1988). We suspect that the phenotypical differences among the three subgroups of rats in the present study are related first of all to genetic differences in emotional responsiveness of rats to this stressful situation.

Good learners were able to overcome their resistance to enter the previously shocked compartment and acquired an adequate instrumental response. Poor learners, in contrast, continued to display an exaggerated emotional reaction, freezing and a restricted response repertoire during the entire first session.

The locomotor and investigatory activity which reappears at the beginning of each session and which is accompanied by several short-latency escape responses suggests that the emotional tension conditioned to the contextual cues during the preceding sessions may dissipate overnight. However, pain from the first grid shocks primes the recollection of this emotional tension, resulting in reinstatement of motionless freezing during ITIs, resistance to reenter the previously shocked compartment and the long-latency "staying" responses we observed at the early trials of each session.

Retention of instrumental defensive responses has been investigated previously in relation to the between-session decrement either after insufficient learning (Kamin 1963) or when a criterion of performance was reached. It was demonstrated that in one-way (Feigley and Spear 1970), two-way active avoidance (Zieliński et al. 1991) and passive avoidance (Spear et al. 1973) alike, avoidance performance markedly decreased after a 24 h intersession interval. It has been suggested that the classically conditioned emotional component is forgotten more slowly than the memory of a specific instrumental response (Feigley and Spear 1970) and that a warm-up effect contributed to reinstatement of the instrumental response (Kamin 1963). Experiments with the use of various warm-up procedures, each involving shock presentation, demonstrated that the improvement in performance after a warm-up treatment was due to facilitation of memory retrieval of the specific task (Spear et al. 1973).

The acquired escape response is instrumental because it terminates the pain action; however, it is ineffective in reducing the emotional tension elicited by the experimental situation. In contrast to the consequences of unpredictable and uncontrollable aversive events (Seligman et al. 1971), the instrumental component of the escape response prevents formation of learned helplessness, and rats retain the incentive for "searching" for a solution to the problem, i.e., how to control the impending shock. The instrumental component of the escape response appears to be responsible for the upper limits of learning in all three subgroups of rats we distinguished on the basis of their performance during the first training session. Escape learning, however, was not monotonic. The multiple oscillations between "direct" and "staying" escape responses may be viewed as attempts to improve the effectiveness of behavior and, consequently, to escape from the threatening experimental situation.

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