ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 1997, 57: 41-47

Changes of intertrial response rate with elapse of time after two-way avoidance trial in rats

Kazimierz Zieliński and **Eugeniusz Nikolaev**

Department of Neurophysiology, Nencki Institute of Experimental Biology, 3 Pasteur St., 02-093 Warsaw, Poland

Abstract. The changes of intertrial response (ITR) rate along the intertrial interval duration were examined in 30 rats trained in two-way avoidance. The lowest ITR rate was observed just after a cross-through response terminating a trial. Rats trained with a warning signal of darkness showed over-all higher ITR rates and shorter periods of post-trial reduction of ITR rate than rats trained with a more salient noise warning signal. The period of post-trial low ITR rate lengthened in consecutive sessions indicating the gradual development of a safety state.

Key words: two-way avoidance, intertrial interval duration, intertrial response rate, fear, safety, stimulus modality, rat

INTRODUCTION

The procedure that is often used in two-way active avoidance training permits a subject to move during intertrial intervals in any direction, so that it may cross away from or back into the compartment in which it had been previously. Such a free-responding situation enables observation of changes of crossing response rate after the termination of the warning signal (conditioned stimulus, CS). The rate of intertrial response (ITR) may be used as an indicator of fear of the contextual cues (Mowrer and Lamoreaux 1951, McAllister and McAllister 1971, Owen et al. 1978, Callen 1986). Pronounced differences in ITR rate between groups of rats trained in two-way avoidance with either noise or darkness as a warning signal have been noted (Zieliński et al. 1991, 1993). The reported data indicate that less fear was conditioned to contextual cues in rats trained with noise, a salient CS, than in rats trained with changes of illumination, a less distinctive CS.

The effects of intertrial interval (ITI) duration on learning and performance were analyzed at early studies of avoidance response acquisition. Experiments on distributed *versus* massed practice showed more rapid avoidance response acquisition and higher resistance to extinction after longer than after shorter ITI durations (Murphy and Miller 1956, Levine and England 1960, Brush et al. 1964). Brush (1962) hypothesized that during the interval between avoidance trials, the intensity of fear decreased asymptotically, and extension of the ITI duration permitted a greater reduction of fear. Thus, longer ITIs should more strongly reinforce the avoidance response.

This notion is supported by some experimental data on the temporal distribution of intertrial responses in two-way avoidance training with the ITI of constant duration. It was shown in dogs taught to jump a barrier in a shuttle-box that at the beginning of training most ITRs occurred immediately after the preceding trial. In contrast, during subsequent extinction sessions, dogs performed more ITRs toward the end of the intertrial interval (Black and Carlson 1959). This change in the distribution of ITR rate could not be related to the withdrawal of shock during extinction sessions, since a similar time discrimination with most ITRs at the end of intertrial intervals of constant duration was observed after prolonged training of two-way escape responses in rats (Matsuyama and Tsukioka 1960). The drift of ITRs towards the end of ITI may be due to the rise of fear related to the anticipation of the next shock (Sidman 1958).

In this study, changes of ITR rate during intertrial interval were analyzed in two groups of rats trained either with a darkness or noise warning signal. In our experiments ITIs of three different durations were presented in a quasi-random sequence. The aim of the present study was to retrace changes of ITR rate within each duration of the ITI independently. Since in well trained rats anticipation of the next trial increases with time, the level of fear and the ITR rate should increase asymptotically reaching the highest level toward the end of the ITI of the longest duration. If, however, a main source of the ITR was a residual fear after a trial (Zieliński 1993), the ITR rate should decrease monotonicaly with time. In this case the trial given after the ITI of the longest duration will start at the low level of fear. The aim of this study is to verify either the residual fear hypothesis or the anticipation of danger hypothesis.

METHODS

Subjects

In this experiment the rules established by the Ethical Committee on Animals Research of the Nencki Institute, based on a decree of the President of Polish Republic, were strictly followed.

Young adult, female Möll-Wistar rats weighing 140-200 g, bred in the Nencki Institute Animal Facility, experimentally naive, were used in this study. Seven or eight animals were kept in a single home cage (43 cm long, 25 cm wide, 18.5 cm high) containing food and water available *ad libitum*. A normal light-dark cycle was induced by natural illumination. Rats were trained once a day in the morning or early afternoon, in the same order and at about the same time each day.

Apparatus

Experiments were conducted in a shuttle-box 62 cm long, 18 cm wide, and 29 cm high with walls of opaque dark acryllic. The box was divided in half by an 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 covered by a movable transparent acryllic ceiling and illuminated by a 5 W lamp mounted centrally just below the 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 cross-through response was recorded by photocells mounted 4 cm to either side of the central partition, 5 cm above the floor level. The direction of a locomotory response was indicated by a succession in which photocells were covered. 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. The shuttle-box apparatus was placed in a dark, sound-proof room. The subjects' behavior was watched on a TV monitor in an adjoining room, where equipment for automatic programming of the experiment and recording of data was located.

Procedure

Prior to the experiment, 30 rats were assigned randomly to two experimental groups, 15 subjects each. Each rat was habituated to the situational cues of the apparatus for 10 min on two consecutive days. On the next day, avoidance training started. At the beginning of a 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; 3 s later, the nominal 1.6 mA scrambled, pulsed DC shock was delivered through the grid-floor (unconditioned stimulus, US). Running to the opposite compartment within the 3 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 coterminated the CS and US and was scored as an escape response. The maximal shock duration was 60 s. Each daily training session consisted of 20 trials. The intertrial intervals were of 20, 30, and 40 s duration and varied in a semi-random order. During the ITI, 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 located at the end of the ITI.

During the nine daily training sessions the groups differed only in the CS used. Group D was trained with darkness as the CS, the Group N was trained with the onset of a 70 dB (re: 20 $\mu N/m^2$) wide band (white) noise presented in the occupied compartment as the CS. The darkness CS was provided by termination of the ceiling light in the compartment occupied by a rat whereas the opposite compartment was illuminated as before. Just after the rat left the shock compartment it was again lit and both compartments were illuminated during intertrial intervals. In Group N both compartments were continuously illuminated throughout session.

Measures

The main measures of behavior were the frequency of avoidance responding, the number of ITR performed after termination of a trial and the latencies of these cross-through responses. The latency of each ITR performed during the actual intertrial interval was measured from the moment of termination of the trial. ITRs were collected and analyzed separately for intertrial intervals of 20, 30, and 40 s ITI duration. The latencies of cross-through responses were measured by computer within 0.01 s accuracy. For statistical analysis and graphical construction, the size of the class interval for avoidance responses was 0.5 s, and for ITRs was 2 s.

RESULTS

Avoidance performance

During the habituation sessions and at the beginning of training, a low level of crossing activity was noted. During the 1st training session only 6% of the trials were terminated with the avoidance response. Thus, during this session the mean probability to perform an avoidance response during each second of isolated CS action was 0.019 in Group N and 0.022 in Group D. Then rapid acquisition of two-way avoidance was observed. At the 2nd training session 70% of rats in each group performed more avoidances than escape responses. The 2 (group) x 9 (day) ANOVA for repeated measures yielded an effect of day [$F(8,224) = 97.90$, $P<0.001$], but not an effect of group, and no interaction. The individual variability of avoidance performance in Group D was greater than to the auditory warning signal. During the 9th session Group N reached higher level of avoidance performance than Group D ($P<0.01$, Kolmogorov-Smirnov two-tailed test).

The evolution of avoidance response latencies during training was investigated by a method used in previous experiments (Zieliński et al. 1993, 1995). For each group the trend of changes of avoidance response latency in the course of training was calculated for different points of the cumulative distribution functions. The percentage of responses emitted with latencies equal to or less than 0.5 s, 1.0 s, 1.5 s, and so on up to 3.0 s was compared for con-

TABLE I

Values of the Spearman rank correlation coefficient between the number of avoidance responses up to and including 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 s in consecutive training sessions and the cumulative number of trials at the end of each session

Time point	Group N		Group D	
	rho	*P*	rho	*P*
0.5 s	0.1035	NS	0.0168	NS
1.0 s	0.5188	NS	0.3025	NS
1.5 s	0.8333	0.01	0.3025	NS
2.0 s	0.9833	0.002	0.5356	NS
2.5 s	0.9958	0.002	0.8285	0.02
3.0 s	0.9833	0.002	0.9121	0.002

secutive sessions. For each time point the Spearman rho correlation coefficient between the group mean percentage of avoidance responses and the cumulative number of trials at the end of the corresponding session was obtained. A positive rho value indicates an increasing, and a negative rho value a decreasing, trend of the percentages of avoidance responses at a given time point. The larger the absolute value of the rho coefficient, the fewer distortions from a monotonic trend were observed in the course of training. Results of such a time-trend analyses are presented in Table I. As is seen, the Spearman rho correlation coefficients were all positive, denoting increases of avoidance performance at all time points of the CS-US interval. The increase of avoidance performance fluctuated at the beginning of the warning signal and was more consistent toward the end of the CS-US interval. The rho values were higher for Group N denoting fewer distortions of avoidance performance increase over training than that observed for Group D.

Intertrial responding

Rats of Group D performed more intertrial responses than rats of Group N and this difference was noted during each training session. The comparison of ITR rate for consecutive sessions suggests a gradual decrease of intertrial responding in Group D and no change in Group N. This trend was confirmed by a 2 (group) x 9 (day) repeated measures ANOVA for the number of ITRs, which yielded effects of group [$F(1,28) = 7.39$, $P<0.01$], day [$F(8,224) = 4.06$, $P<0.001$], and their interaction [$F(8,224) = 3.13$, $P<0.004$].

A more detailed analysis of ITR rate was based on data collected separately for ITIs of 20 s, 30 s, or 40 s duration. Examination of the records revealed pronounced individual variability of the ITR rate and fluctuation of this index from session to session for nearly each subject. Thus, the numbers of ITRs were summed within a given ITI duration for each group and block of three consecutive sessions to smooth out the records. Then the mean ITR rate for each consecutive 2 s fragment of the ITI was calculated. The results for Group D are presented in Fig. 1 and for Group N - in Fig. 2.

A low ITR rate was observed just after termination of the trial by an instrumental response (the 0-2 s fragment) in each group and stage of training. During the early training sessions rats trained with the darkness CS increased their ITR rate during the following dozen or so seconds reaching maximum at the end of the shortest, 20 s ITI. In Group D the period of low ITR rate extended in the course of training up to 12 s during the block of 7-9 sessions. Maximal ITR rate observed in the vicinity of the 20 s ITI duration also decreased somewhat at the last sessions. Both of these changes were responsible for a

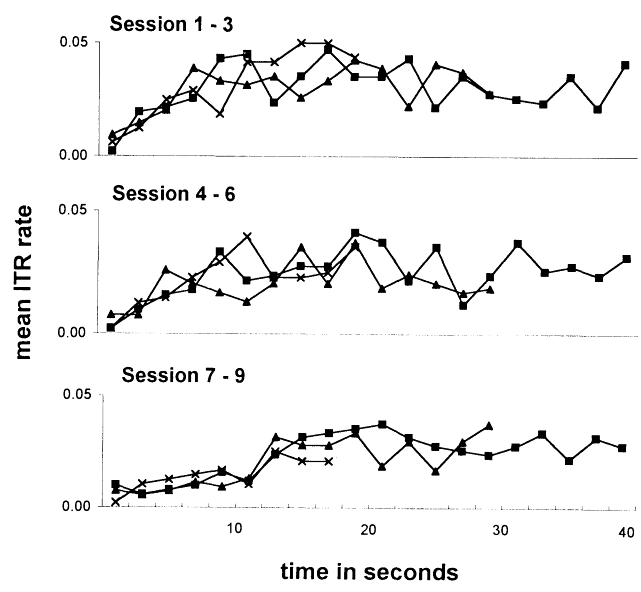

Fig. 1. The rate of intertrial responses in Group D for consecutive 2 s fragments of the 20 s (crosses), 30 s (triangles), and 40 s (squares) intertrial intervals during blocks of three consecutive sessions: Session 1-3 (upper row), Session 4-6 (middle row), and Session 4-6 (bottom row). The ITR rate denote probability of ITR incidence in one s for an averaged rat of a group depending on a 2 s fragment of a given ITI duration and a block of sessions.

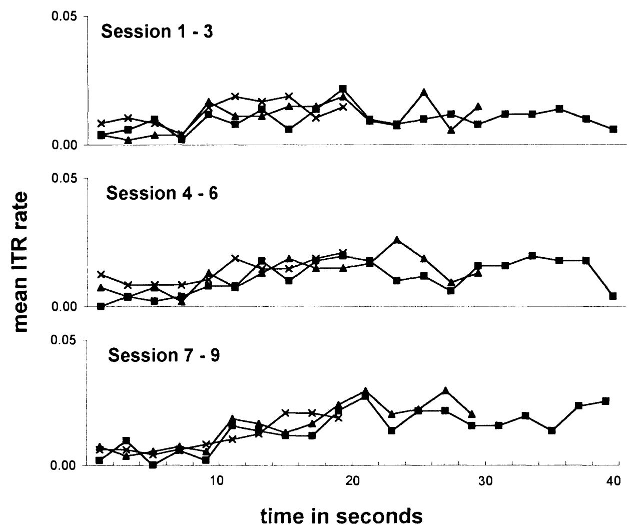

Fig. 2. The rate of intertrial responses in Group N for consecutive 2 s fragments of the 20 s, 30 s, and 40 s intertrial intervals during blocks of three consecutive sessions (denotations as in Fig. 1).

gradual decrease of over-all ITR rate over the consecutive sessions in Group D.

The period of low ITR rate after termination of the trial was longer in rats trained with noise (Group N). At the beginning of training it lasted for 8 s. A rather small but abrupt increase of ITR rate was observed during the subsequent duration of the ITI.

All of those inferences were confirmed by repeated measures ANOVA based on mean ITR rate for a group of rats and three session block calculated for each ITI duration independently. Results of ten such ANOVAs for each 2 s fragment of the ITI up to 20 s are presented in Table II. No differences in the 0-2 s fragment were observed. During the next 2 s fragment a decrease of ITR rate in consecutive blocks of sessions was evident, which was more pronounced in Group D. A block effect was significant from 2.01 s up to 12 s and a group effect from 4.01 s to the 20 s. The interaction of the two variables was noted sporadically.

DISCUSSION

In the present experiment the effect of stimulus modality on avoidance performance was rather small. Only toward the end of prolonged training, did the rats perform more avoidance responses to the noise warning signal than to the darkness CS. Special tests revealed, however, a more regular rise of avoidance performance in rats trained with noise than with darkness. The effect of stimulus modality on avoidance performance was demonstrated previously both in between-subject (Jacobs and LoLordo 1977, Zieliński et al. 1991, 1993) and within-subject (Zieliński et al. 1995) designed experiments. Presumably short CS-US interval, long ITI, and a small number of trials within a session were all responsible for rapid avoidance acquisition and also for attenuation of the stimulus modality effect on avoidance performance.

In accordance with earlier results (Zieliński et al. 1991, 1993), the ITR rate was lower in rats trained with a noise warning signal and higher in rats trained with darkness. Our attention was focused on the distribution of ITR within the intertrial interval and changes of the ITR rate over training.

TABLE II

Results of the repeated measures analysis of mean intertrial response rate for groups (Group N vs., Group D) and blocks (1-3, 4-6, and 7-9 sessions) done independently for each 2 s fragment of the intetrial interval up to 20 s. In the body of the Table *F* values are presented

	Upper limit of the ITI period									
	2	4	6	8	10	12	14	16	18	20
Group	0.00	5.18	76.78*	74.95*	13.34*	9.55*	45.68*	37.34*	38.06*	107.61*
Block	1.09	5.63*	5.60*	11.00*	8.93*	5.00*	1.17	0.49	3.98	0.90
Interaction	2.05	6.76*	1.47	21.02*	1.19	6.87*	1.56	1.02	9.46*	3.35

The values of degree of freedom for Group was (1,4), for Block (2,8), and for Interaction (2,8); *denote significance at $P<0.05$ level.

Changes of the ITR rate reflect fluctuations of fear during intertrial interval. In the present experiment, the distribution of ITRs along the intertrial interval indicates a strong reduction of excitation by termination of CS with an instrumental response. However, such a reduction was not complete. The ITR rate gradually increased along the ITI duration denoting the recovery of fear with the flow of time. The maximal ITR rate was reached toward the 20 s point and did not increase in the course of longer ITIs. This finding suggests the relation of a gradual increase of ITR rate to the anticipation of the forthcoming trial and possible shock application.

Experimental data on ITR distribution within the intertrial interval are highly controversial. Results obtained on dogs (Black and Carlson 1959) and rats (Matsuyama and Tsukioka 1960) trained in two-way avoidance with an ITI of fixed duration were in agreement with the hypothesis that the main source of intertrial responding was residual fear from a trial (either avoidance or escape) which gradually diminished and became replaced by anticipation of a forthcoming trial. Cats trained in bar-press avoidance executed most of the ITRs soon after termination of the trial. The hypothesis of residual fear was additionally supported by the highest ITR rate just after delayed termination of a CS and also after shock trials, when the avoidance response was not performed (Zieliński and Plewako 1980). The U-shaped temporal distribution with peaks immediately before and after shock was noted in rats terminating the shock with a bar press (Badia and Culbertson 1970). The highest ITR rate before the next trial was observed in dogs during avoidance extinction (Black and Carlson 1959) and in rats overtrained in unsignalled avoidance (Sidman 1958). In the present experiment, the lowest ITR rate just after the trial termination was observed from the very beginning of training. Presumably, such a pattern of ITR rate changes may be typical for overtrained subjects, or when the required instrumental response is taken from the repertoire of species specific defensive responses as it is with running from a dangerous place in case of rats (Bolles 1970).

A recent experiment in which the CER method and a forward defensive conditioning procedure were employed showed that the suppression of appetitive responding during the defensive CS was immediately followed by enhancement of on-going behavior above the baseline after termination of a trial (Walasek et al. 1995). The high rate of bar pressing response for food reward after a defensive trial continued for a substantial portion of the ITI and then decayed together with the rise of expectancy of the forthcoming trial and shock US. This post-trial enhancement of the appetitive behavior may indicate that the temporal and contextual stimuli are becoming a safety signal after termination of the defensive forward trial.

The two-way avoidance response trained in a shuttle-box consists in leaving the shock compartment and entering the safe compartment. If the warning signal may be easily discriminated from the experimental context, the environmental and temporal stimuli immediately following US (shock) never closely precede the next US. Thus, they may acquire properties of a fear inhibititor (Moscovitch and LoLordo 1968, Siegel and Domjan 1971, 1974, Ayres et al. 1976). The present experiment showed that discrimination between the warning signal and the environmental and temporal stimuli following the shock US was easier with noise, a salient CS, than with darkness, a weaker and less distinctive CS. The noise warning signal resulted not only in a more consistent increase of avoidance performance, but also in the longer period of reduced post-trial ITR rate.

Cross-through responses performed during ITI had no consequence for the subsequent warning signal onset. Thus, from the point of view of energy expenditure the ITRs should be considered maladaptive. However, some behavioral and physiological observations suggested that the ITRs not only reflected a changeable fear level but also served to decrease emotional tension of a subject (Zieliński 1993).

ACKNOWLEDGEMENTS

The authors appreciate useful suggestions by Dr. W. Ławicka to earlier version of the paper and careful verification of the English style by Dr. K. Turlejski and an unknown referee. This study was supported by a statutable grant from the State Committee for Scientific Research to the Nencki Institute.

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Received 18 September 1996, accepted 27 December 1996