ATTAINMENT AND STABILITY OF THE PERFORMANCE IN DIFFERENTIAL LOW RATE WATER REINFORCEMENT IN RATS

Haruyoshi OGAWA, Hisashi KURIBARA, Kiyoko OKUIZUMI and Sakutaro TADOKORO
Behavior Research Institute, School of Medicine, Gunma University, Maebashi 371, Japan
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Abstract—The establishment and stability of the behavioral baseline for rats in relation to the schedule of differential reinforcement of low rate under water reinforcer (DRL 20 sec for water) were studied, with the following results: When the DRL value was gradually stepped up from 1 sec to 20 sec with the advance of the sessions from 1 to 16, the establishment of the behavioral baseline was slower than when DRL 20 sec was applied from the start. The establishment of the baseline was clearly accelerated by the prolongation of the length of time for training in one session from 60 min to 120 min. The baseline remained highly stable without being affected by the intermittent administration (2-3 times a week) of methamphetamine and diazepam, each in doses from 0.06 to 1.0 mg/kg, and of caffeine and pentobarbital, each in doses from 1.2 to 20 mg/kg, or by the discontinuation of the test from 1 to 15 days. However, during the retraining period following the test discontinuation it was found that the baseline fluctuated for a long time due to the elimination of water deprivation. The baseline stability, once established, could be maintained through about 300 daily sessions, with only a slight dependence on the change in environmental conditions such as humidity, temperature, and the season.

Though it goes without saying that the kind of drugs, the doses, and the time-course of drug effects cannot be disregarded in the evaluation of the effect of psychotropic drugs on operant behavior, it is well known that another important determinant is the characteristic of the schedule of reinforcement for the observation of the behavior, especially the rate of lever-pressing before drug administration (1). From this viewpoint, the schedule of differential reinforcement of low rate (DRL schedule), in which the rate of lever-pressing is low, gives the investigator maximum advantage in evaluating such drugs as those which elevate the rate. Food pellets are widely employed as a reinforcer in this schedule, and the psychopharmacological problems in such cases have been investigated in detail (2, 3). On the other hand, water, which is indispensable for life, is more economical than the pellet, is easier to handle and can be considered most useful as a reinforcer. However, for the practical application of a water reinforcer to the performance under the DRL schedule, it is necessary to perform systematic studies concerning long maintenance of the performance.

The object of the present work was to investigate the fundamental problems in training by the DRL schedule under water reinforcer, with regard to number of training sessions, the various skills necessary in establishing the behavioral baseline and maintaining its
stability, and pharmacological application of this schedule.

MATERIALS AND METHODS

Experimental animals

The animals used were Wistar strain male rats, weighing 280–330 g, which were 90 days old at the start of the experiment. They were fed laboratory food—MF (Oriental Yeast Co., Tokyo). As for drinking water, the following limitation was enforced.

Water deprivation

The rats were deprived of water to such an extent as to decrease the body weight to about 80% that of normal. Thus about 7 ml/rat of tap water was given immediately after the termination of the experiment. This amount was consumed within 30 min, but no more was provided until the next experiment. On the days of no experiment, 15 ml/rat/day was given, since about 10 ml/rat was consumed in the test chamber during the operant schedule. As a rule, the water deprivation was maintained throughout, except when studying the effect of elimination of the deprivation.

Apparatus

A tray of drinking water was placed near a lever, which was set on the internal wall of a Skinner-box. Each time a rat correctly pressed the lever according to the schedule, a solenoid valve operated for a moment, and about 0.15 ml of water flowed onto the tray from a water bottle.

The Skinner-box was placed in a sound-proofed, ventilated box, and the general behavior of the rat was observed in another room through a TV monitor.

Schedule-programming

The object of the DRL schedule is to make a rat correctly perform temporally spaced lever-pressings. It is so programmed that the lever-pressings will not be reinforced so long as the interresponse time is shorter than a certain fixed interval. Thus, in case of DRL 20 sec, the rat must wait at least 20 sec after the last lever-pressing. If the lever-pressing occurs within those 20 sec, the timer is instantly reset, and the animal is forced to wait another 20 sec. Furthermore, the length of time allowed for the effective lever-pressing (namely, the pressing which can be reinforced) is limited to 60 sec; and if this time is passed without any lever-pressing, the timer is again reset. In other words, only the first lever-pressing within the effective time zones can be reinforced.

Grouping and training

The rats numbered 25 in all, and were divided into 5 groups of 5 each—A, B, C, D and E—according to the object of the observation. With groups A, B and C, the number of training sessions required for establishment of the behavioral baseline was observed; and with groups D and E, the effects of the administration of various psychotropic drugs, and of test discontinuation, respectively, were investigated in relation to the stability of the established baseline.

The training session was usually carried out once daily, and was so continued daily.
The length of one session was 60 min for groups A, B, D and E, and 120 min for group C.

To groups A, C, D and E, DRL 1 sec was applied only in the first 10 trials of the 1st session, and then increased at once to DRL 20 sec, which was later maintained throughout. In group B only, the DRL value was gradually stepped up from 1 sec to 20 sec during the early sessions of the training period. That is, rats were trained on DRL 1 sec in the 1st and 2nd session, on DRL 2 sec in the 3rd-5th session, on DRL 5 sec in the 6th-8th session, on DRL 7 sec in the 9th-11th session, on DRL 10 sec in the 12th-15th session, and finally on DRL 20 sec in the 16th session. The last DRL value was maintained thereafter.

Psychotropic drugs

The drugs used were methamphetamine hydrochloride (0.06-1.0 mg/kg), caffeine (1.2-20 mg/kg), diazepam (0.06-1.0 mg/kg) and pentobarbital sodium (1.2-20 mg/kg). All were administered subcutaneously.

Environmental conditions

The temperature in the home cage was kept at 20-25°C throughout the year, and that in the Skinner-box at 25-30°C. As for the change in the humidity and the season, no special precautions were taken.

Calculation of response rate and percentage of correct responses

The number of lever-pressings per 10 min was calculated from the total number of lever-pressings in one session and this was designated as the response rate. The percentage of correct responses indicates the ratio of the number of effective lever-pressings to the total number of lever-pressings. The response rate and the percentage of correct responses are shown on the ordinates, and the session number on the abscissas, and for the curves thus obtained, the slopes of the regression lines were calculated by the method of least squares from the points in each section of 10 sessions. These slopes were used as indices of attainment of DRL performance and of stability of the baseline, respectively.

RESULTS

I. Differences due to training method in the speed of establishment of the behavioral baseline

1) Stepping-up of DRL value

Group A: Fig. 1 compares cumulative records of lever-pressings in the 3rd, 4th, 5th, 10th, 20th and 40th session on the same coordinate. The slope of the curve gradually decreased as the sessions increased. Especially in the early sessions, the curve fluctuated widely, owing to irregular lever-pressings, and the frequency of reinforcement was low. In the 40th session, however, the slope of the curve was gentle, presenting a high linearity and regular and frequent distribution of diagonal strokes which indicated reinforcements. The cumulative records after the 40th session were essentially identical to the record for the 40th session.

Fig. 2 was obtained by plotting the mean response rate and the mean percentage of correct responses for each session from the start of training to the 100th session. In general, the response rate tended to be higher and the percentage of correct responses lower in the
early sessions of training. With advance in session, however, the response rate gradually lowered, and the percentage of correct responses was conversely elevated; after the 50th session, both indices remained almost stable. In this way, the establishment of the behavioral baseline was confirmed. At this time, the response rate was about 35/10 min, and the percentage of correct responses about 35%.

Fig. 1. Cumulative records of lever-pressings of a rat in group A. The diagonal strokes indicate reinforcements.

Fig. 2. Session-to-session changes in response rate and percentage of correct responses from the 1st to the 100th session in group A.

Acquisition and Maintenance of the Behavior of DRL 20 SEC for Water
After the start of the deprivation, the body weight declined continuously for about 3 weeks, but later remained at an almost constant level.

**Group B:** Fig. 3 compares the results from group A with those from group B as the DRL value was gradually stepped up from 1 sec to 20 sec in the latter group. The response rate increased radically at each step-up, while at the same time the percentage of correct responses were markedly low, therefore presenting, on the graph, wide fluctuation.

In association with the step-up of DRL value, marked irregularity was observed in the stereotyped behavior (for example, nibbling of the tail, i.e. collateral behavior) occurring between two instances of the lever-pressing.

Fig. 4 represents the processes of the establishment of the behavioral baseline after the 16th session for groups A and B. The mean response rate and the percentage of correct responses fluctuated far more markedly in group B than in group A, and the slope of the regression line was steeper in B. Moreover, group B required about 80 sessions of training for the stabilization of the behavioral baseline; thus the establishment of the baseline in group B was markedly delayed as compared with that in group A. After the 80th session, the response rate and the percentage of correct responses in group B were about 33/10 min and 40% respectively, which were not so notably different from those in group A.

Body weight change from the deprivation in group B was nearly the same as in group A, and in both groups, 2 to 3 weeks were required to attain the constant level.

![Fig. 3](image-url) Changes in response rate and percentage of correct responses of groups A and B in early sessions of training. The figures above or below solid dots indicate the stepped-up DRL values.
Fig. 4. The process of establishment of baselines after the 16th session in groups A and B. The solid lines were drawn connecting the midpoints of successive regression lines, which were calculated by the method of least squares dividing into sections of 10 sessions.

Fig. 5. The process of establishment of baselines by prolongation of the length of training time in one session (group A; one session = 60 min, group C; one session = 120 min). Response rate and percentage of correct responses were divided into two time zones in group C (middle column; 0–120 min, lower column; 0–60 min).
2) **Prolongation of the length of time for training in one session**

When training time of 120 min per one session and DRL 20 sec were applied from the 1st session to group C, the establishment of the baseline was apparently accelerated as compared with the case of group A in which the training time of one session was 60 min. Fig. 5 indicates changes in the response rate and the percentage of correct responses of group C with which those in group A were contrasted, dividing into two time zones designated as 0-60 min and 0-120 min. The final value of the response rate was evident after about the 10th session and that of the percentage of correct responses was seen after about the 30th session in group C without reference to the time zones.

II. **Stability of the behavioral baseline**

1) **Influence of drug effect**

With all drugs used in this experiment, the response rate was increased and the percentage of correct responses was decreased. Differences were manifested in the magnitude of drug effect depending upon the type of drug and the dose given. A maximum effect was shown after the administration of methamphetamine (doses over 0.5 mg/kg), and it was about a two-fold increase in the response rate and about a 90% decrease in the percentage of correct responses when compared with the baseline. Fig. 6 represents the baseline after intermittent administration of the above-mentioned drugs. The drugs were usually given 2 or 3 times a week at the position indicated by the wedge-shaped mark "V". The results in the drug administered sessions were excluded from the graph and have been described elsewhere.

![Behavioral Baseline after Administrations of Methamphetamine (0.06 - 1 mg/kg), Caffeine (1.2 - 20 mg/kg), Diazepam (0.06 - 1 mg/kg), and Pentobarbital (1.2 - 20 mg/kg). Group D](image)

**Fig. 6.** Behavioral baseline after administrations of methamphetamine (0.06-1 mg/kg), caffeine (1.2-20 mg/kg), diazepam (0.06-1 mg/kg) and pentobarbital (1.2-20 mg/kg). The solid lines were calculated by the method of least squares from the points in each section of 10 sessions.
(4, 5), since the purpose of this experiment was not to demonstrate the drug effects themselves but rather to illustrate whether or not influence of those effects still remained on the baseline. Descriptions of the order of drug administration were also omitted from the graph, since there was little correlation between the magnitude of the drug effect and changes in the baseline. In Fig. 6, it is clearly shown that the drugs would not appreciably affect the solid lines. Thus the effects of drugs used in this experiment were reversible and the baselines were stable regardless of frequent administrations.

2) Influence of test discontinuation

In Fig. 2, 1–3 day test discontinuations are indicated by the arrows, and in Fig. 6, 15 day discontinuation is denoted by an asterisk "*". These figures show that a marked fluctuation in the response rate and the percentage of correct responses never occurred for more than two sessions. Thus the test discontinuation ranging from 1 to 15 days did not produce any serious distortion on the baseline so long as the water deprivation was strictly maintained.

Fig. 7 represents shifts in the response rate and the percentage of correct responses produced by the 30-day test discontinuation with removal of the water deprivation in group E in which the baseline had already been established. In this case, the response rate returned to the pre-discontinuation level in about 10 sessions after the resumption of the test, but fluctuation in the percentage of correct responses persisted for about 40 sessions. Along with this, remarkable differences were observed in the slopes of regression lines. Thus in the case of test discontinuation with removal of water deprivation, many sessions were required in order to re-establish the baseline.

Fig. 8 indicates changes in body weight in the same sessions as demonstrated in Fig. 7. After the resumption of water deprivation, decrease in body weight was still seen for about 40 sessions and the final value of the weight (about 25% decrease) was shown thereafter.

After the resumption of the test, marked irregularity was observed in the pattern of

![DISCONTINUATION OF TEST FOR 30 DAYS WITHOUT WATER DEPRIVATION](image)

**Fig. 7.** Effects of the test discontinuation with removal of water deprivation in regard to the re-establishment of the baseline. The solid lines were calculated by the method of least squares from the points in each section of 10 sessions.
collateral behavior accompanying the lever-pressing, and the restoration of the initial pattern required almost the same length of time as the re-establishment of the baseline.

**DISCUSSION**

If *DRL* performance, which is characterized by a low rate of lever-pressing, can be maintained using water as well as food reinforcement, the application of *DRL* schedule to psychopharmacological tests will be simplified and made easier because of the economic, manipulative and other advantages of water.

The results in the present experiment pointed out three problems, which will be discussed herein. The first is the fact that the establishment of the behavioral baseline required as many as 50-80 sessions of training when the training time of one session was 60 min. In order to substantially shorten the training period, it would seem reasonable to lengthen the time of training in each session to more than 60 min. This can be easily determined from the fact that the establishment of the baseline was earliest in group C, for which the length of one session was 120 min.

The second is the fact that when the *DRL* value was stepped up gradually, contrary to anticipation, the establishment of the baseline was markedly delayed. According to Wilson and Keller (6) and Weiss and Laties (7), the effective lever-pressing on *DRL* schedule is largely dependent upon collateral behavior. From this view point, it is considered that collateral behavior became markedly irregular immediately after the step-up of *DRL* value. On the other hand, the training showed good results in the absence of the step-up, when the collateral behavior was maintained at a constant pattern.

The third is the fact that when the deprivation was removed during the test discontinuation, the re-establishment of the baseline required many sessions of training. In regard to this problem it is considered that the rats were again affected remarkably by water de-

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**Fig. 8.** Changes in body weight after resumption of water deprivation. The deprivation was initiated after 2:00 p.m. on the last day of test discontinuation.
privation after the test was re-started and thereby the tendency for an increase in the number of the lever-pressings ensued to comply with the level of deprivation. Actually, it has been noted that there is a close relationship between the frequency of lever-pressings on DRL schedule and the level of water deprivation (8). This can also be applied to the problem proposed above. Furthermore, it is indicated that water deprivation resulted in a decrease of body weight under free feeding conditions (9, 10). In the present experiment, we observed that body weight decreased gradually during the training period which followed a discontinuation of the test in group E. Thus it seems that the close relationship between frequency of lever-pressings and water deprivation may be expressed in terms of body weight loss. It is therefore strongly recommended that the deprivation should be continued throughout the period of test discontinuation so as to maintain the body weight at a certain low level.

We have thus outlined the problems encountered when water reinforcer was used to determine the performance under DRL 20 sec. However, none of these problems is considered inherent or attributable to water reinforcement.

It can be concluded from the results of the present experiments and discussion that use of water reinforcer is feasible for pharmacological determinations of the DRL schedule. Moreover, the results make it clear that with adequate precautions, the baseline of the DRL performance maintained by water reinforcer is stable for about one year, and this baseline can be used as the basis for the assay of the effects of drugs.

REFERENCES

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