ANALYTICAL STUDY OF ACQUISITION ON FREE-OPERANT AVOIDANCE RESPONSE FOR EVALUATION OF PSYCHOTROPIC DRUGS IN RATS*

Hisashi KURIBARA, Kiyoko OKUIZUMI and Sakutaro TADOKORO

Behavior Research Institute, School of Medicine, Gunma University, Maebashi 371, Japan

Accepted July 14, 1975

Abstract—Systematic research was performed on the acquisition of free-operant (Sidman-type) avoidance response, which is widely used for the study of psychotropic drugs, with the following results: When the training session was fixed at 2 hours once daily, shock-shock (S-S) interval at 5 seconds, and response-shock (R-S) interval variable—20, 30 and 60 seconds, the acquisition speed of the response was almost constant independent of the R-S interval, about 6 sessions being always required. When the S-S and R-S intervals were constantly 5 and 30 seconds, respectively and the length of one session was varying—1, 2 and 4 hours, the behavioral baseline was established after about 6 sessions independently of the length of the session. Thus the cumulative time for the acquisition was shortest when one session was 1 hour long, and longest when it was 4 hours long. There was a linear relation with negative inclination between the logarithm of mean numbers of shock delivered per session and the number of sessions. In rapid and exact training of animals for the evaluation of drug effects, one session of an hour per day is adequate. In the evaluation of drug effects on the acquisition process, observation of the shift in logarithmic value of shocks delivered is recommended.

The free-operant (Sidman-type) avoidance (1) is one form of operant behavior most extensively used for the evaluation of psychotropic drugs. As for the practical method of animal training, workers’ views are varying, and there is yet no consensus (2–7).

The authors want to propose an adequate method of animal training, and in addition to analyse the regularity between the acquisition progress and the number of shocks delivered, in order to emphasize the pharmacological applicability of this relation.

MATERIALS AND METHODS

Subjects: Wistar strain male rats, bred by brother-sister mating for more than 20 years in the Department of Pharmacology were used. They were fed a solid diet of MF (Oriental Co. Tokyo) and given tap water ad libitum. At the start of training, the animals were age 70–100 days and weighed 250–300 g.

Thirty-five rats were divided into 7 groups of 5 according to experimental conditions. Except for the period of experiment, the groups were separately maintained in their respective cages.

* Supported by a special fund from The Science and Technology Agency of Japan for promoting multiministrial projects 1973–1975 on “The studies on regeneration of nervous tissue”
Table 1. Grouping of rats and experimental conditions

| Experiment | Group | R-S interval (sec) | S-S interval (sec) | Length of session (hr) | No. of rats |
|------------|-------|-------------------|--------------------|-----------------------|-------------|
| A          | I     | 20                | 5                  | 2                     | 5           |
|            | II    | 30                |                    |                       |             |
|            | III   | 60                |                    |                       |             |
| B          | IV    |                   |                    |                       |             |
|            | V     | 30                | 5                  | 0.5                   |             |
|            | VI    |                   |                    | 1                     | 5           |
|            | VII   |                   |                    | 2                     |             |

Table 1 summarizes experimental conditions as classified by group. The experiments were grossly divided, according to purpose, into A and B. In A, the effects of the difference in R-S interval on the acquisition process were investigated, while the object of B was to find the most efficient length of training session, which is also related to the learning ability of the animal.

The training was performed, as a rule, every other day from 8:00 a.m. – 8:00 p.m., and no rat had more than one training session a day. The same Skinner-box and programming system were used throughout the experiment.

**Apparatus:** In the Skinner-box, 35 cm wide, 20 cm long and 22 cm high, the floor is made of a grid, consisting of 22 stainless steel rods, 4 mm in diameter, arranged parallel to the width, 1.5 cm apart on center. Electric current can be passed through the grid by means of a shock scrambler.

A stainless steel lever is set in the middle of a side wall, 5.0 cm over the grid. It is 3.5 cm wide, and 3.0 cm long. When a rat presses the lever downward with power of more than 15 g, a microswitch attached to the lever will operate, generating 0.5 sec pulses, and the response (lever pressing) becomes effective.

For the prevention of noise, the Skinner-box was placed in a wooden sound-proof box, 50 cm wide, 35 cm long and 50 cm high. During the training, the box was illuminated with a 10W white fluorescent lamp, set on the middle of the ceiling of the sound-proof box, and the air was kept fresh with a ventilator in the wall. The temperature in the box was controlled at 23±2°C. The programming and recording systems were placed in the next room.

**Programming:** In the present schedule, electric shock by 225 V, 2 mA, 50 cycle AC was periodically (S-S interval) delivered for 0.5 sec when the animal did not press the lever. But each time the lever was pressed, the shock was postponed for a certain time (R-S interval). Consequently, if the lever pressing was repeated at appropriate intervals, that is, shorter than the R-S interval, the shock could be avoided.

**RESULTS**

**Effects of R-S interval on the acquisition**

Fig. 1 summarizes the results of training of groups I–III, with the S-S interval and the length of session being fixed at 5 sec and 2 hr, respectively, and with the R-S interval being
ACQUISITION OF AVOIDANCE RESPONSE

Effects of R-S interval on the acquisition

S-S interval and training length per session were fixed to 5 sec and 2 hr respectively. R-S interval was varied 20, 30 and 60 sec.

a. Frequency of responses
b. Number of delivered shocks

varied (20, 30 and 60 sec). Herein were plotted the mean numbers of responses and shocks per 10 min for each training in 1–10 sessions. In general, the results was unsatisfactory for 10–20 min after the start of training in each session, and thereafter a gradual improvement was seen. As a consequence, the data obtained in the first 30 min of training were eliminated from the calculation of the mean value, since they were considered to represent the warm-up effect and not the true state of acquisition. The mean numbers of responses tended to increase with the progress of training until about 5 sessions. This tendency was the more marked, the shorter the R-S.

On the other hand, the number of shocks decreased with an increase in the number of sessions. This tendency was especially remarkable up to 5–6 sessions, and was not affected by a difference in R-S interval, all resulting in the exponential curve shown in Fig. 1-b. Thus, judging from the decrease in the number of shocks and their later stability, the acquisition process is considered unrelated to the R-S interval.

Effects of length of session on the acquisition

Fig. 2 represents the acquisition process for groups IV–VII with the S-S and R-S intervals being fixed at 5 and 30 sec, respectively, and with the length of one session being varied (0.5, 1, 2 and 4 hr). In conformity with the shortest session (0.5 hr), mean numbers of responses and shocks per 10 min for the first 30 min of training were plotted in 1–10 sessions. The mean numbers of responses and shocks attained about 50/10 min and 10/10 min, respectively, in about 5 sessions regardless of the length of the sessions. Thereafter, these numbers remained stable with no significant differences between groups, although the data for the first 30 min showed the warm-up effect as described above.
FIG. 2. Effects of length of a session on the acquisition.
S-S and R-S intervals were fixed at 5 and 30 sec respectively. Length of a session was varied 0.5, 1, 2 and 4 hr. In conformity with the shortest session (0.5 hr), each mean value was determined by the results for the first 30 min of training.
a. Frequency of responses
b. Number of delivered shocks

FIG. 3. Effects of length of a session on the acquisition.
Experimental conditions as in Fig. 2. Mean values for the 30–60 min period were plotted in conformity with the length of session for the 1 hr group.
a. Frequency of responses
b. Number of delivered shocks

In Fig. 3, the mean values for the 30–60 min period were plotted in conformity with the length of session for group V (1 hr). Also in this case, there was no significant difference between groups. The mean number of responses and shocks attained about 60/10 min and 3/10 min, respectively, after several sessions and thereafter remained nearly stable. These results indicate that the acquisition speed was not affected by the length of a session.

Analytical study of acquisition

For a more quantitative description of the acquisition speed, the authors attempted mathematical analysis of the change in the number of shocks delivered. As seen in Figs. 1
and 3, the mean number of shocks exponentially decreased with each repetition of session. When the logarithmic values of the mean number of shocks in Exp. A were taken on the ordinate and number of sessions on the abscissa, the graph shown in Fig. 4 was obtained. Fig. 4 shows that the state of acquisition is expressed by two straight lines of different inclinations on the two different sides of 5 sessions, which thus marks a turning point. The straight lines in the figure were determined by the method of least square in 1–5 sessions and 6–10 sessions. The figures beside the lines denote the decrease rate of mean numbers of shocks. There is no significant difference between groups in the decrease rate in 1–5 sessions, that is, the inclination of the straight line. But the decrease rates in 6–10 sessions are evidently smaller than those in 1–5 sessions. Only the inclination for group III seems exceptionally greater, but the change in absolute numbers of shocks is very small as seen in Fig. 1-b. Thus it was indicated that in any training method, rapid acquisition might be realized within 5 sessions. This has important significance in the study of the learning process.

Fig. 5 was obtained by taking the cumulative training hours on the abscissa regardless of
FIG. 5. Relationship between logarithmic value of delivered shocks and cumulative training hours.
S–S and R–S intervals were fixed to 5 and 30 sec respectively. Each point was plotted every 30 min. Values obtained in the first 30 min of training were eliminated.

a. 1 hr-session group
b. 2 hr-session group
c. 4 hr-session group

The session, and the logarithmic number of shocks on the ordinate on the basis of results of Exp. B. In this case, the data for the first 30 min of each session, which reflect the warm-up effect, were eliminated. In conformity with the 1 hr session for group V, the cumulative training time was divided into 30 min intervals. A relatively linear relation was maintained up to 5 sessions after the start of training. The straight lines and their inclinations were determined by the method of least square. There was some scatter after 6 sessions, but the general tendencies can be shown by these expressions.

The figures beside the straight lines denote the mean decrease rates of the number of shocks per hour. There is an approximate inverse proportion between training hours of a session and the inclinations of the line. Thus the inclinations for the 2- and 4-hr training groups were about 1/2 and 1/4, respectively, of those for the 1-hr group. This means that the acquisition should be decided rather by the number of sessions regardless of the cumulative hours of training when the length of each session ranges 1–4 hr.

DISCUSSION

The free-operant (Sidman-type) avoidance response is also termed “nondiscriminated
avoidance response” or “continuous avoidance response” (1). This method, which requires relatively easier antecedent operation and simpler experimental apparatus than any other operant schedule and which can establish a behavioral baseline or accomplish the learning in a relatively shorter time, is used in psychopharmacological experiments with rats, especially in assaying such neuroleptics as phenothiazine and butyrophenone derivatives.

According to Cook and Kelleher (8) and Janssen et al. (9, 10), there is a very close correlation between inhibitory effect on the avoidance response and antipsychotic effects. And it has been confirmed in many reports (11–13) and summarized in many reviews (8, 14–17) that the avoidance response specifically reflects the inhibitory effect of neuroleptics. In view of this, the avoidance test is now regarded as one of the indispensable pre-clinical trials for the psychotropic drugs.

Usually this test is used to assay the drug effects by observing changes in response rate, number of shocks delivered, interresponse time, response latency, number of escape failures, lever holding time, etc. (18, 19). In this case, rats sufficiently well trained to show a constant behavioral baseline are used, since the drug effects often vary according to the degree of training of the animal. The authors (20) previously reported that the effects of chlorpromazine and methamphetamine on the discriminated avoidance response of rats varied with their grade of skill and training. Sacks et al. (21) reported that chlordiazepoxide, which generally exerts an inhibitory effect on avoidance response after the completion of learning, conversely showed an accelerative effect in the same dose when the training was at the halfway point.

On account of this, it has become mandatory for the exact assay of drug effects, not only to stabilize the behavioral baseline, that is, to train animals effectively and exactly, but also to make a detailed analysis of the acquisition process. Furthermore, a concrete method of training for numerous well-fitted animals in the shortest possible time can be established.

Kelleher and Morse (22) and Dews (23) advanced the view that both quantitative and qualitative change in the drug effects on operant behavior would sometimes be affected by the frequency of responses. Thus, the inhibitory effect is generally enhanced in case of high response rate, and the accelerative effect in case of low response rate. In fact, the same drug exerted opposite effects depending on the response rate (19, 21). The free-operant avoidance schedule is convenient in this respect, since a convenient response rate can be freely determined by changing the R-S interval.

It is also possible to train animals more efficiently by fixing the training time of each session to 1 hr, and by repeating the training at 2–3 day intervals. In this case, the behavioral baseline will be established within 10 sessions. Tadokoro (24), one of the authors, reported that not only in the free-operant avoidance schedule but also in most of the other avoidance schedules, the interval between sessions exerted little effect on the acquisition, and that after more than 100 days of training interruption following the establishment of a behavioral baseline, the baseline will be recovered within 30 min of retraining at the longest. After stabilization of the baseline by 1 hr training in each session, the authors’ study
showed, that lengthening of the session to 2–4 hr and more did not exert any effect on the baseline. These results indicate that once the behavioral baseline is established in rats after 1 hr training in each session, the animals can be used, without retraining, in the long-time assay of drug effects, and that the acquisition will be preserved for the whole life of the rat.

The mathematical analysis of the present results disclosed that there is a linear relation, having a negative inclination, between the logarithm of the numbers of shocks delivered and the numbers of sessions during the acquisition process. And the inclination of the line, which is assumed to express the acquisition speed, was nearly constant regardless of the R-S interval. These facts provide useful indicators for the assay of the drug effects on the learning process, and will find extensive application.

There are many reports (18, 24, 25) on the effect of drugs on the learning process, but the majority are based on qualitative investigations alone. According to the present analysis, the above discussed schedule can be applied to the study of drug effects upon memory and to the quantitative assay of drug effects on acquisition speed, which has hitherto been considered difficult.

REFERENCES

1) Sidman, M.: Operant Behavior: Areas of Research and Application, Edited by Honig, W.K., p. 448, Appleton-Century-Croft, New York (1966)
2) Sidman, M.: Science 118, 157 (1953)
3) Sidman, M.: J. comp. physiol. Psychol. 46, 253 (1953)
4) Dinsmoor, J.A.: Psychol. Rev. 61, 42 (1954)
5) Verhave, T.: J. exp. Anal. Behav. 2, 185 (1959)
6) Anger, D.: J. exp. Anal. Behav. 6, 477 (1963)
7) Hurwitz, H.M.B.: J. exp. Anal. Behav. 10, 55 (1967)
8) Cook, L. and Kelleher, R.T.: A. Rev. Pharmacol. 3, 205 (1963)
9) Janssen, P.A.J., Niemeggers, C.J.E. and Scheltekens, K.H.L.: Arzneim. Forsch. 15, 114 (1965)
10) Janssen, P.A.J., Niemeggers, C.J.E. and Scheltekens, K.H.L.: Arzneim. Forsch. 15, 1196 (1965)
11) Courvoisier, S., Fournel, J., Ducrot, R., Kolsky, M. and Koetschet, P.: Archs int. Pharmacodyn. Thér. 92, 305 (1953)
12) Cook, L. and Weidley, E.: Ann. N. Y. Acad. Sci. 66, 740 (1957)
13) Heise, G.A. and Bolt, E.: Psychopharmacol. 3, 264 (1962)
14) Dews, P.B. and Morse, W.H.: A. Rev. Pharmacol. 1, 145 (1961)
15) Hunt, H.F.: A. Rev. Pharmacol. 1, 125 (1961)
16) Steinberg, H., De Reuck, A.V.S. and Knight, J.: Animal Behavior and Drug Action, Churchill, London (1964)
17) Goldur, L.R. and Brady, J.V.: A. Rev. Pharmacol. 5, 235 (1965)
18) Niemeggers, C.J.E., Verbruggen, F.J. and Janssen, P.A.J.: Psychopharmacol. 16, 161 (1969)
19) Takaori, S., Yada, N. and Mori, G.: Japan. J. Pharmacol. 19, 587 (1969)
20) Tadokoro, S., Okuzumi, K., Kuribara, H. and Ogawa, H.: Folia Pharmacol. Japon. 70, 67p (1974) (in Japanese)
21) Sachs, E., Weingarten, M. and Kielin, N.W. Jr.: Psychopharmacol. 9, 17 (1966)
22) Kelleher, R.T. and Morse, W.H.: Ergebn. Physiol. 60, 1 (1968)
23) Dews, P.B.: Fedn Proc. 17, 1024 (1958)
24) Tadokoro, S.: Folia Pharmacol. Japon. 67, 135p (1971) (in Japanese)
25) Ader, R. and Clinck, D.: J. Pharmacol. exp. Ther. 121, 144 (1957)