EFFECTS OF CENTRAL DEPRESSANTS ON ROTA-ROD AND TRACTION PERFORMANCES IN MICE

Hisashi KURIBARA, Yoichiro HIGUCHI
and Sakutaro TADOKORO
Behavior Research Institute, School of Medicine,
Gunma University, Maebashi 371, Japan
Accepted September 27, 1976

Abstract—Effects of central depressants—chlorpromazine, diazepam, pentobarbital and ethanol—on rota-rod and traction performances in mice were investigated. The walking technique of the animals on the rotating rod (3 cm in diameter, 24 r. p. m.) was established after about 15 trials, and was well maintained for one week thereafter. No training was required for the traction. Both the rota-rod and traction performances were inhibited by chlorpromazine, diazepam, pentobarbital and ethanol in fairly good parallel with the dosages. However, the sensitivities to one same drug markedly differed between the two performances. Chlorpromazine more than diazepam inhibited the rota-rod, while in the traction performance the inhibition with diazepam was greater than that with chlorpromazine. Pentobarbital and ethanol inhibited the two performances to nearly the same degree. On the basis of the present results, the rota-rod test is considered to be suitable for the estimation of the positive adaptability to forced motor activity, and the traction for that of muscle relaxation. By comparing the dose-effect relationships in the two performances, it may be possible to elucidate the characteristics of various central depressants, and to apply these procedures for the screening test in drug evaluations.

When a mouse is repeatedly placed on a rod or cylinder which is rotating at a constant speed, the animal gradually learns to walk on it, adapting itself to the rotation speed. After ingestion of a central depressant, however, the animal easily falls from the rod. This procedure is called ‘rota-rod test’ and was first introduced by Dunham and Miya (1) for assaying the drug effects on the motor activity. Since then, the effects of various central depressants, investigated by this test have been reported (2-4). There is, however, no established interpretation of the results obtained by this test, for many factors, e.g. lowered general activity, ataxia, muscle relaxation, fatigue, etc, are considered to inhibit the rota-rod performance (RR performance).

On the other hand, when a mouse hangs from a thin bar by means of its forelimbs, the animal can maintain this posture for a certain time without falling. This traction behavior (TR performance) or grasping reflex is also sharply altered by central depressants.

We studied the effects of various central depressants on these two performances, and found that the sensitivities differed depending on the drug.

MATERIALS AND METHODS

Adult, male ddG strain mice were obtained from the breeding room of Gunma University, Medical School. A solid diet MF (Oriental Yeast Co., Tokyo) and tap water were
provided ad libitum. These animals were used for experiments at the age of 40–60 days, at which time the body weight had attained 25–30 g. About 600 mice were used for the RR test, and about 300 for the TR test.

Drugs used included chlorpromazine hydrochloride (CPZ) (Contomin Inj., Yoshitomi), diazepam (DZ) (Cercine Inj., Takeda), pentobarbital sodium (PB) (Mintal Inj., Tanabe) and ethanol (EtOH). Immediately before the administration, the drug was diluted with saline, a 20% propylene glycol (PG) or distilled water to adjust to a single dose to 0.1 ml/10 g body weight. CPZ, DZ and PB were given s.c., and EtOH i.p. The doses of the drug were graded into 4–6, the smallest hardly effective, while the largest completely inhibited both performances. Control animals were given the corresponding volumes of the solvent alone.

Apparatus and procedure

RR test: A rota-rod treadmill for mice (Ugo Basile, Italy) was used. A plastic rod, 3 cm in diameter, and 30 cm long, with non-slippery surface and 15 cm over the base was used. This rod is divided into 5 equal sections by 6 discs, thus enabling 5 mice to walk on the rod at the same time. In the present experiment, the speed of 24 r.p.m. only was used. Intervals between the mounting of the animal on the rod and falling off of it were recorded as the performance time. The result was assessed by the 6-score criteria shown in Table 1.

The training of mice by RR test was given 20 times at 5–15 min intervals. Thereafter, 60 mice were randomly selected to determine the retention of the walking technique. Other animals who performed on the rod for more than 10 sec were used for assaying the drug effects immediately after the training. The remaining animals showing poor results were excluded from all further experiments.

After the administration of the drug, the performance time was measured at 10 min intervals for 60–90 min. For the assay of each one dose, 20–25 trained mice were used, and repetition of the drug test was never made with one same animal.

TR test: A stainless steel bar, 1.5 mm in diameter with the horizontal part measuring 7 cm, was hung 30 cm over the base. A mouse hung from the horizontal part by the forelimbs, and after confirming that the grasp was good, it was left hanging, and the time when the animal fell was recorded.

The pre-drug trial was repeated two or three times, and animals hanging on for more than 10 sec were used for the assay of drug effects. As in the case of RR test, TR performance time was observed at 10 min intervals for 60–90 min. For the assay of each dose, 10 mice were used. Repetition of drug assay in the same animal was avoided.

RESULTS

RR and TR training and performance

Training process by RR test: Table 1 shows the distribution of performance times of 300 mice given 20 trials of training at 10 min intervals. The column on the far right gives the criteria for the assessment. About 25% of all mice were excluded from the drug test because their performance times were less than 10 sec after 20 trials of training. The average score for 300 mice in the 20th trial was 4.05, and that for 227 after excluding 73 mice with
TABLE 1 Distribution of times of rota-rod performance after 20 trials

| Time (sec) | No. of mice | Distribution (%) | Score |
|-----------|-------------|------------------|-------|
| 0– 4      | 32          | 10.7             | 0     |
| 5– 9      | 41          | 13.7             | 1     |
| 10– 14    | 26          | 8.7              | 2     |
| 15– 19    | 11          | 3.7              | 3     |
| 20– 24    | 19          | 6.3              | 4     |
| 25– 29    | 13          | 4.3              | 5     |
| 30– 49    | 27          | 9.0              | 6     |
| 50– 99    | 43          | 14.3             | 6     |
| 100–199   | 54          | 18.0             | 6     |
| 200–299   | 11          | 3.7              | 6     |
| 300       | 23          | 7.7              | 6     |

Fig. 1 Acquisition process of the rota-rod performance as expressed by means of performance time and score. One session consisted of 20 trials in a day, and the second session was carried out one week after the first. The ranking criteria are shown in Table 1. Upper: Mean performance time. Lower: Mean performance score. Vertical lines indicate standard error.
poor results was 5.17.

Fig. 1 represents change in the performance as shown by average time and score for another 60 mice in 20 trials. The performance of 300 sec was taken as the upper limit in this figure, though some mice remained on the rod more than 600 sec. Animals in this experiment were again subjected to the same training after one week, and the results were compared with those of the first session.

In the 1st trial of the first session, no animal could deftly walk on the rod, and fell within 2 or 3 sec. With the repetition of the trial, however, the walking was progressively prolonged, and after about 15 trials, the mean performance time and score attained to about 60 sec and 4, respectively. Later the performance was almost stabilized. The values in the 20th trial were approx the same as those for the 300 cases in Table 1. In the second session of training, the walking was possible from the 1st trial, and in the 15th and later trials, the mean performance time and score were about 80 sec and 4.3, respectively. Comparison of training processes in the first and second sessions revealed that the mean performance times

![Graph showing performance changes](image_url)

**Fig. 2** Temporal change in scores of the RR and TR performances after chlorpromazine. Upper: Scores of RR performance. Lower: Scores of TR performance. Filled marks indicate the significant difference from the saline administered control value (p<0.05).
in the 1st-12th, and the mean scores in the 1st-7th and the 10th trials were significantly higher in the latter than in the former (p<0.05). It was also found that mice producing poor results in the first session showed little improvement in the second.

Training process by TR test: In the TR test, few mice fell within 10 sec even in the 1st trial, and most animals could cling to the bar more than 30 sec. Thus, the performance needed no training. The average score for 300 mice as obtained by the same criteria as in RR test (Table 1) was 5.74.

Drug effects

Effects of chlorpromazine: Fig. 2 represents temporal changes in RR and TR performances through a 90 min period after CPZ administrations. The ordinate is the average score and the abscissa the time after the administration. The filled marks indicate a significant difference from the saline administered control value (p<0.05), (Student's t-test).

CPZ in a dose of 0.125 mg/kg exerted little effect on RR performance, however, when the dose was increased, the performance was inhibited in fairly good parallel with the doses, and the maximum effect was produced at about 50 min after the administration. The

![Graphs of ROTA-ROD and TRACTION performances](image)

**Fig. 3** Temporal change in scores of the RR and TR performances after diazepam (expressed in the same way as Fig. 2). Control animals were given a 20% propylene glycol.
performance was gradually restored to the saline administered control level. CPZ elicited a similar pattern of change in TR performance to that in RR. There was, however, a great difference in the drug sensitivities between RR and TR, and in terms of dose ratio, RR was about 10 times as sensitive as TR.

Effects of diazepam: Fig. 3 represents the effects of DZ on RR and TR performances in the course of 90 min, as in Fig. 2. In the PG administered control group, no change was observed in either performance. When DZ was given in doses over 1 mg/kg, evident inhibition of RR performance was manifested immediately after the administration, and the effect was enhanced and prolonged in fairly good parallel with the doses. The effect of DZ on TR performance showed a similar pattern of change to that seen with RR, but the effects of DZ were more evident with the TR than with the RR. Thus, in the present experiment, the result of DZ was the reverse of CPZ.

Effects of pentobarbital: Fig. 4 represents the effects of PB on RR and TR performances in the course of 90 min. When the dose was over 10 mg/kg, inhibition of both performances was manifested immediately after the administration. The intensity and duration of the effect were increased almost in proportion with the doses. When the dose
was over 30 mg/kg, the righting reflex was abolished in some animals at about 10 min after the administration. This continued until about 30 min, when both RR and TR performances were completely inhibited. Recovery of the righting reflex, in both performances tended toward restoration.

Effects of ethanol: Fig. 5 represents temporal changes in the effects of EtOH on RR and TR performances in the course of 60 min after administration. When the dose was over 1 ml/kg, the inhibitory effect of EtOH was evident immediately, and the intensity and duration increased in fairly good parallel with the doses. In a dose of 4 ml/kg, EtOH abolished the righting reflex in almost all cases, and such was not restored within one hour.

Summary of the drug effects on RR and TR performances: Fig. 6 represents, in the same coordinate, the dose-effect curves of CPZ, DZ, PB and EtOH on RR and TR performances. In this figure, the average scores of 30–60 min after the drug administrations are expressed as a percentage to the pre-drugged score.

![Diagram of Rota-Rod and Traction performances](image)

**Fig. 5** Temporal change in scores of the RR and TR performances after ethanol given i.p. (expressed in the same way as Fig. 2 except that the observation period was 60 min).
According to the drugs given, large differences in the sensitivities between RR and TR were evident. Thus, the inhibitory effect of CPZ on RR performance was stronger than that on TR, while the effect of DZ was just the reverse. PB and EtOH inhibited both performances to almost the same degree. Similar dose-effect relationships could be also obtained in other observation periods.

**DISCUSSION**

There are many methods to assay drugs which affect motor coordination in animals. The positional sense test, righting test, gait and stance test, muscle tone test and equilibrium test have all been used (5, 6). The RR test was first introduced by Dunham and Miya (1) as a screening test to assay the neurotoxicity of anticonvulsants, and later was reported to predict motor dysfunction produced by central acting drugs (2–4). Recently this test has been used as one of the pre-clinical routine tests for psychotropic drugs (7).

As for the significance of the RR test, different workers stated different views, and comparisons with results obtained by other methods has often been attempted. The most
prevalent practice is the measurement of spontaneous motor activity, and there are many reports on the effects of neuroleptics (2, 8, 9), benzodiazepines (9, 10) and barbiturates (2). Sometimes the inclined screen method (9, 10) is also used for the object of comparison with the muscle-relaxing effects.

The present experiment demonstrated clearly that RR performance can be established by training, and that once acquired, the walking technique on the rod could be maintained for one week. About 75\% of all animals given the training could be used for the assay of the drug. On the other hand, TR performance proved a 'natural' to the mice, subsequently, almost all mice could be used without previous training.

Standardization of the RR and TR procedures remains a challenge. It is necessary in the RR test, for example, to standardize the materials, diameter and rotation speed of the rod, and the upper limit of the performance time, all of which seem to subtly affect the result of drug assay. Plotnikoff et al (4) reported the influence of the rotation speed, and Watzman et al (11) discussed significance of the rod's diameter and rotation speed. Jones and Roberts (12), and Watzman and Barry (13) proposed a method to estimate the drug effect from the rotation speed, which was gradually elevated until the animal fell.

Since RR performance is considered to be an acquired behavior dependent on skill, it is possible that drug tolerance, state dependence and enhancing drug effect (14–16) may be induced by the repetition of drug administration. Repeated use of the same animal for the drug test should be carefully avoided.

The RR and TR performances changed according to the type of drug. Comparison of the dose-effect relationships on the two performances revealed that central depressants may be classified into the following three types: 1) CPZ type; inhibits RR performance to a much greater extent than it does the TR performance. 2) DZ type; inhibits TR performance to a much greater extent than it does the RR performance. 3) PB or EtOH type; inhibits the two performances to almost the same degree.

It is assumed that the inhibition of RR performance elicited by CPZ is due to a lack of positive adaptability to forced motor activity than to dysbasia, equilibrium disturbance, muscle relaxation or catalepsy. This is, in some respects, identical with the inhibitory effect on the conditioned avoidance response. Actually, the temporal change of inhibition elicited by CPZ on the conditioned avoidance response in rats (17) was quite similar to that in RR performance in mice.

The inhibitory effect of DZ on TR performance is considered to reflect the muscle-relaxing effect. RR performance is often maintained for a long time even in animals in which ataxia is observed after DZ or PB administration. In consideration of this fact alone, muscle relaxation cannot be regarded as a main causative factor of inhibition of RR performance.

In the present pharmacological aspect, CPZ is classified as a major tranquilizer or neuroleptic drug, DZ as a minor tranquilizer or antianxiety drug, and PB and EtOH as a general CNS depressant. Their respective properties, as suggested by the above classification, were confirmed by the present experiment. This has an important significance,
since the methods employed herein can be applied in the screening test in the drug evaluations. The tests can be carried out easily, because the apparatus and procedures are relatively simple, and the breeding condition of animals need not be absolutely strict.

REFERENCES

1) DUNHAM, N.W. AND MIYA, T.S.: J. Am. Pharmacol. Assoc. 46, 208 (1957)
2) KINNARD, W.J. JR. AND CARR, C.J.: J. Pharmacol. exp. Ther. 121, 354 (1957)
3) GLUCKMAN, M.I.: Curr. Ther. Res. 7, 721 (1965)
4) PLOTNIKOFF, N., REINKE, D. AND FITZLOFF, J.: J. Pharmacol. Sci. 51, 1007 (1962)
5) SWINYARD, L.A., BROWN, W.C. AND GOODMAN, L.S.: J. Pharmacol. exp. Ther. 106, 319 (1952)
6) HARNED, B.K.: J. Pharmacol. exp. Ther. 107, 403 (1953)
7) MAXWELL, D.R.: Psychopharmacology, Dimensions and Perspectives, Edited by JOYCE, C.R.B., p. 57, Tavistock Publications, London (1968)
8) WEAVER, J.E. AND MIYA, T.S.: J. Pharmacol. Sci. 50, 910 (1961)
9) NAKAMURA, K., ZBINDEN, G. AND RANDALL, L.O.: Pharmacology of Psychotropic Drugs, Asakura, Tokyo (1971) (in Japanese)
10) ZBINDEN, G. AND RANDALL, L.O.: Pharmacology of Benzodiazepines, Laboratory and Clinical Correlations, Advances in Pharmacology, Vol. 5, p. 213, Academic Press Inc., New York (1967)
11) WATZMAN, N., BARRY, H. III, KINNARD, W.J. AND BUCKLEY, J.P.: Archs int. Pharmacodyn. Ther. 169, 362 (1967)
12) JONES, B.J. AND ROBERTS, D.J.: J. Pharm. Pharmacol. 20, 302 (1968)
13) WATZMAN, N. AND BARRY, H. III: Psychopharmacol. 12, 414 (1968)
14) TADOKORO, S., OGAWA, H., OHASHI, K., KANAZAWA, Y., KONISHI, T. AND MURATA, M.: Japan. J. clin. Pharmacol. 2, 356 (1971) (in Japanese)
15) TADOKORO, S., OGAWA, H., SHIBAZAKI, M., OHASHI, K., SHIROTA, M., HIRABAYASHI, M. AND IZUKA, M.: Folia pharmacol. japon. 71, 68P (1975) (in Japanese)
16) HIRABAYASHI, M., SHIBAZAKI, M., IZUKA, M. AND TADOKORO, S.: Folia pharmacol. japon. 71, 126P (1975) (in Japanese)
17) TADOKORO, S.: Basic Pharmacol. Ther. 2, 1412 (1974) (in Japanese)