STUDIES OF THE SPONTANEOUS MOVEMENT OF ANIMALS BY THE HOLE CROSS TEST; EFFECT OF 2-DIMETHYLAMINOETHANOL AND ITS ACYL ESTERS ON THE CENTRAL NERVOUS SYSTEM

Keijiro TAKAGI, Minoru WATANABE* and Hiroshi SAITO
Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo
Received for publication July 5, 1971

It is the first and important practice for the study of the psychotropic drugs to observe the spontaneous movement of animals. Although many methods have been known for measuring the spontaneous movements, most of them are to find the change of motor activity of a single animal. Many investigators reported the automatic measurement of the motor activity. Photoelectric method was first used by Siegel et al. (1), which was improved by Dews et al. (2) in mice. Takagi et al. (3) traced the spontaneous movement of a mouse using a modified photoelectric apparatus.

The central nervous system stimulant effect of 2-dimethylaminoethanol (DMAE) was first reported by Pfeiffer et al. (4) and had been confirmed by Konigsmark et al. (5), Himwich (6) and Brown and Gangloff (7). It was also confirmed by clinical trials. Pfeiffer et al. (8) proposed the hypothesis that DMAE played the role of a precursor of acetylcholine in the central nervous system. Groth et al. (9) found that DMAE was incorporated in the mouse brain more rapidly than choline and probably converted to brain choline. However, Pepeu et al. (10) and Kiplinger et al. (11) had different views from that of Pfeiffer.

In this paper, we have developed a method of recording the spontaneous activity of grouped mice, in order to take an accurate measurement of a little change of motor activity by drugs. We investigated the effect of DMAE and its acyl esters on the spontaneous movement of the grouped and/or individual mice.

MATERIALS AND METHODS

1) Hole cross test in mice

A steel partition is fixed in the middle of a cage, 30 × 20 × 14 cm. A hole of 3 cm in diameter, is made at the height of 7.5 cm in the centre of the plate (Type I) and a mouse can pass through it only one at a time. A light and a phototransistor are set up in the opposite positions of the hole at a height of 7.5 cm and the same apparatus on the other side of the plate. When a mouse passes through the hole, two horizontal light beams running along the hole at both sides of the plate, are cut off at the same time. It is immediately counted and

* Present address: Department of Toxicology and Pharmacology, Research Institute for Chemical Hazards, Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo.
recorded electromagnetically on the kymograph, and the total motor activity and the time course of motor activity in the mice can be analysed. The electric circuit of this apparatus is shown in Fig. 1.

Ten mice were put simultaneously in the cage and observed for 2 hours. The experiment started 3 times a day at 10:00, 12:30, and 15:00. Three groups of ten mice were allotted to 3 (day) \times 3 (time) Latin square designs. Only the difference between the times were significant as shown in Table 1. Total motor activity at the first time of the day was about twice more than those at the remaining two times, that is, the average number of passing

**Table 1. Latin square design of the hole cross test of Type I.**

| Time | B1 10:00 | B2 12:00 | B3 15:00 | t     | Group | t    |
|------|---------|---------|---------|-------|-------|------|
| Day  |         |         |         |       |       |      |
| A1   | C1 1308 | C2 523  | C3 441  | 2272  | C1    | 2389 |
| A2   | C1 1085 | C2 719  | C3 482  | 2286  | C2    | 2130 |
| A3   | C1 1212 | C2 599  | C3 522  | 2333  | C3    | 2372 |
| t    | 3805    | 1481    | 1445    | T 6891|       |      |

**Analysis of variance table**

| Factor | SS  | Df | Ms  | Fcal  |
|--------|-----|----|-----|-------|
| A      | 0.06| 2  | 0.03|       |
| B      | 89.37| 2 | 45.69| 24.00 > P = 0.05 |
| C      | 1.06| 2  | 0.53|       |
| D      | 3.73| 2  | 1.86|       |
| Total  | 8   |    |     |       |

The result of one of 18 groups was shown.
through the hole of 18 groups, which were allotted to 6 different Latin square designs, resulted in 1101 ± 30, 525 ± 28, and 485 ± 24 (frequency ± 2.E.). The peak effect was found within 30 min and after 2 hours the mice became quiet (Fig. 2a).

In the second experiment three groups of each mice were tried repeatedly at the same time of every day, and the trials were continued for 7 days. The alteration of the total motor activity and time course of motor activity of the mice were not seen among the 7 days. The result was shown in Fig. 3.

**Fig. 2.** Effects of drugs on the change of the time course of motor activity in mice.

Ten mice were put simultaneously in the cage 5 minutes after administration of drug.
FIG. 3. Effect of DMAE on motor activity in mice given once a day 1 hour before the hole cross test of type I for 7 days.

*significant at $P<0.05$

** significant at $P<0.02$

Ordinate: % change of motor activity was taken as the ratio of the total motor activity of the test to that of the control trial.

These data show the average of 6 groups.

Because the total motor activity and the time course of motor activity were not stabilized in groups of mice weighing 12-15 g, mice weighing 18-22 g were used in this experiment. The room temperature was kept at $22\pm2^\circ C$ in all trials.

For testing the effect of a drug, saline was at first used on the first day as a control, and the drug was tested at the same time of the 4th day. The motor activity index was taken as the ratio of the total activity of the test to that of the control trial.

We also made an apparatus (Type II) having a hole at the height of 1.5 cm in the centre of the plate, and compared it on the motor activity of the grouped mice to that of at the height of 7.5 cm.

2) Phototransistor type recorder in a mouse

This method is described in detail by Takagi et al. (3).

3) Climbing test in mice

This method is described in detail by Sandberg (12).

4) Drugs tested

Methamphetamine was used as hydrochloride, caffeine as sodium benzoate, chlor-

![Chemical Structures of Acylesters of DMAE used.](image)

Fig. 4. Chemical Structures of Acylesters of DMAE used.
promazine as hydrochloride, reserpine (Serpasil), and DMAE as tartrate. Acyl esters of DMAE were synthetized by the method of Schneider (13). The chemical structure of DMAE derivatives tested are given in Fig. 4. All drugs tested were injected intraperitoneally.

5) Animals used

Male mice (dd-strain) weighing 18–22 g were used.

RESULTS

1) Methamphetamine

In the case of phototransistor type recorder, the minimum dosage to increase the motor activity of single mouse is over 0.5 mg/kg intraperitoneally. The results were given in Fig. 5c. This was compared with the effective doses of methamphetamine on the grouped mice in the hole cross test.

The dosage injected intraperitoneally were 0.01–10 mg/kg and saline was given as a control. Ten mice were put simultaneously in the cage five minutes after the administration of methamphetamine. The spontaneous movements in mice injected with methamphetamine increased immediately. In the hole cross test of type I, even the motor activity of mice given 0.01 mg/kg showed a significant increase. The mice given 0.5 mg/kg moved so hard that no decline of peak effect could be observed within 2 hours. It is shown in Fig. 2b. The motor activity of the grouped mice was recognized to increase up to 0.4 mg/kg and gradually decreased as the doses increased. The results were shown in Fig. 5a. In type II, the motor activity of the mice given 0.05 mg/kg showed a significant increase. The peak effect was shown at 10 mg/kg and gradually decreased as the doses increased. It is shown in Fig. 5b.

2) Caffeine

The minimum dosage to get an increase in the motor activity of single mouse is over 2 mg/kg in the phototransistor type recorder. The results were shown in Fig. 5c. In the case of the grouped mice in type I, 0.2–2 mg/kg of caffeine were given and the results were shown in Fig. 5a. The spontaneous movement of the mice injected caffeine was increased 30 minutes after the injection. The duration of the elevated motor activity by caffeine was shorter than by methamphetamine, and two peaks could be recognized in the time course of motor activity. It is shown in Fig. 2c. The motor activity in mice given 0.2 mg/kg showed a significant increase. The peak effect was shown when 4 mg/kg of caffeine was administered. In the test of type II, the motor activity in mice given 1 mg/kg showed a significant increase. The peak effect was shown when 20 mg/kg of caffeine was administered.

3) Reserpine

The climbine test and the hole cross test were tried in mice given 0.01–1 mg/kg of reserpine 2 hours before the test. The results were shown in Fig. 6. In the climbing test, ten mice were put simultaneously in the cage and we observed the number of animals having climbed the wire net ladder 4 times each for 10 minutes starting at 140, 170, 200, and 230 minutes after the injection. The results were shown in Fig. 6b. The significant decrease was obtained on the dose of 0.05 mg/kg, but in the hole cross test of type I, even the administration of 0.01 mg/kg decreased their spontaneous movement significantly. It is shown in
Fig. 5. Effects of methamphetamine and caffeine on motor activity in mice.
* significant at P<0.05
** significant at P<0.02
*** significant at P<0.01
Abscissa: log dose (mg/kg) of drugs.
Ordinate: percent of total motor activity of the mice in comparison with that of control mice as 100%.
These data show the average of 6 groups.
Fig. 6a. In the latter, the decrease of the spontaneous movements was obtained with smaller doses of the drug than those in the former. The spontaneous movements of reserpinized mice disappeared within 30 minutes after the animals were placed in the cage.

4) Chlorpromazine (CPZ)

Chlorpromazine was given to mice in 0.01–2 mg/kg 1 hour before the test. The results were shown in Fig. 6. In the climbing test a significant decrease was obtained in the dose of 0.05 mg/kg (Fig. 6b), but in the hole cross test of type I, in the dose of 0.01 mg/kg (Fig. 6a).
Fig. 6. Effects of reserpine and chlorpromazine on motor activity in mice.

* significant at $P<0.05$

** significant at $P<0.02$

*** significant at $P<0.01$

These data show the average of 6 groups.
5) DMAE and its acyl esters

DMAE is known as an antidepressant and the central stimulating effect is recognized when it is given successively (4). Takagi et al. (3) also observed an increase in the motor activity in the single mouse given DMAE tartrate 100 mg/kg intraperitoneally with caffeine, once a day for a week. We investigated whether the increase in a spontaneous movement in the mouse could be obtained by the hole cross test at a single administration of DMAE and its acyl esters.

5-1) DMAE

DMAE was given in doses of 2, 1, 0.5, 0.25, and 0.125 mmole/kg in the climbing test and the hole cross test of type I and type II. The results were shown in Fig. 7. In the climbing test, we observed the number of animals reaching the top of the ladder during 10

Fig. 7. Effect of DMAE on motor activity in mice.
* significant at P<0.05
** significant at P<0.02
*** significant at P<0.01
These data show the average of 6 groups.
minutes starting at 20, 50, 80, and 110 minutes after the injection. The spontaneous movement of mice given with each of lower three doses of DMAE was as same as that of the control whereas the mice given 1 and 2 mmole/kg decreased their spontaneous movements significantly (Fig. 7c).

In the hole cross test of type I, ten mice given with 0.5 mmole/kg of DMAE were immediately put in the test cage and the time course were analysed. The results were shown in Fig. 2d. After the administration of DMAE the animals were depressed during the first 1 hour. Two peaks were seen in the time course of motor activity and the first peak was smaller than that which we could see in the control. The second one had something like that observed in the case of caffeine, but the peak was smaller and appeared later than that of caffeine (Fig. 2d). So, 1 hour after the administration of DMAE the mice were put in the cage, the total motor activity and the time course were analysed. In this case, only one peak was seen in the hole cross test. In the hole cross test of type I, on significant difference of motor activity from the control could be recognized when 0.125, 0.25, and 0.5 mmole/kg of DMAE were given, though the administration of these doses led somewhat to the increase of the motor activity in mice. As doses were increased, the motor activity of mice decreased obviously (Fig. 7a).

The significant increase of the motor activity in the grouped mice given 0.25 mmole/kg of DMAE 1 hour before the test once a day for a week, was also observed (Fig. 3).

In the case of the hole cross test of type II, no significant difference of motor activity and the time course from the control could be recognized. The results were shown in Fig. 7b.

5-2) Acyl esters of DMAE

Acyl esters of DMAE were given in doses of 2, 1, 0.5, and 0.25 mmole/kg in the climbing test and the results were shown in Fig. 8. The spontaneous movement of the mice given 0.25 mg/kg of acyl esters was as same as that of the control and the mice given each of higher three doses of esters decreased their spontaneous movement. After the administration of esters, the animals decreased their explorative movement during the first 1 hour. Among

![Fig. 8. Effects of acyl esters of DMAE on motor activity in mice in the climbing test.](image-url)
lower acyl esters, the longer the acyl group became, the weaker the depressive activity was, and it attained the minimum at hexanoate of DMAE. When the acyl group became larger than the hexanoyl group, it was increased again.

Acyl esters of DMAE were given in 0.25, 0.5 and 1 mmole/kg in the hole cross test of type I. The results were shown in Fig. 9. By administration of 0.25 and 0.5 mmole/kg of these esters, significant increase of motor activity was observed in the grouped mice given hexanoate, valerate, butyrate and acetate of DMAE. The administration of 1 mmole/kg of acyl esters led the mice to depress the total motor activity obviously, especially acetate, butyrate, octanoate and phenylacetate of DMAE. No significant increase could be recognized when 1 mmole/kg of hexanoate and valerate of DMAE were given, but they led somewhat to the increase in total motor activity. The effect of propionate, heptanoate octanoate and phenylacetate of DMAE on the motor activity had some similarities to that of DMAE.

5-3) Effects on reserpinized mice

Ten mice which had been administered 0.2 mg/kg of reserpine 3 hours before the test and 2, 1, 0.5, 0.25, and 0.125 mmole/kg of DMAE and its acetate and propionate 1 hour before the test, were put in the climbing cage. In the case of the administrations of 0.25 and 0.5 mmole/kg of DMAE and its acetate, the recovery of the decreased spontaneous movement of the reserpinized animals could be definitely observed, and as the dose was increased it was weakened and disappeared. When propionate of DMAE was administered an incomplete recovery of the movement could be obtained only at the dose of 0.25 mmole/kg. The results were shown in Fig. 10.

![Fig. 9. Effects of DMAE and its acyl esters on motor activity in mice by the hole cross test (Type I).](image)

* significant at $P<0.05$
** significant at $P<0.02$
*** significant at $P<0.01$

These data show the average of 6 groups.
FIG. 10. Effects of DMAE, Ac-DMAE and Pr-DMAE on motor activity in mice pretreated with reserpine 0.2 mg/kg i.p. (Climbing test).

* significant at P<0.05
** significant at P<0.02
*** significant at P<0.01

These data show the average of 6 groups.

TABLE 2. Effects of DMAE, Ac-DMAE and Pr-DMAE on motor activity in mice pretreated with reserpine 0.2 mg/kg i.p. (Hole cross test of Type I).

| Compound mmol/kg i.p. | Climbing test | Hole cross test |
|-----------------------|--------------|----------------|
|                       | No. of groups | % change of motor activity | No. of groups | % change of motor activity |
| Control               | 6            | 38±3.0          | 6            | 20±3.0          |
| DMAE, 0.25            | 6            | 60±3.5**        | 6            | 36±3.0**        |
| Ac-DMAE, 0.25         | 6            | 72±4.0***       | 6            | 43±3.5***       |
| Pr-DMAE, 0.25         | 6            | 46±5.5          | 6            | 35±4.0**        |

* significant at P<0.05
** significant at P<0.02
*** significant at P<0.01

These data show the average of 6 groups.

The hole cross test of type I and the climbing test were carried out in the same condition of mice given 0.25 mmol/kg of these drugs. The results were shown in Table 2. Among DMAE, its acetate and propionate, the difference of the total motor activity between the control and the acetate treated animals was most significant. The recovery of the decreased spontaneous movement of the reserpine treated mice could also be found when 0.25 mmol/kg of DMAE and its propionate were given in the hole cross test of type I.

DISCUSSION

It was demonstrated by Chance (14) that the toxicity of amphetamine to mice was considerably higher when tested on grouped mice kept together in one cage or container than
on individual mice kept in separate ones. This aggregation effect may also potentiate central stimulant action of some drugs, and so we prepared the hole cross apparatus. The influence of drugs on the motor activity of the mouse, could be recognized by this test in such a small dose as they could not be detected by the phototransistor type recorder (3) and the climbing test. The significant difference of the motor activity from the control, could be seen by the hole cross test of type I in a smaller dose than by that of type II, though in the case of type I the total activity was reduced to about three quarters in the type II.

The stimulant action of methamphetamine on the grouped mice could be found at the dosage of one fiftieth as small as that found in a single mouse, and caffeine was effective at one tenth. Reserpine and CPZ decreased the motor activity of the grouped mice and a decrease can be recognized at the smaller doses than those in the climbing test.

The effect of drugs on the time course of motor activity can also be obtained by this test. States of excitation of the mice could be seen immediately after the administration of methamphetamine, about 30 minutes after the administration of caffeine and about 1 hour after the administration of DMAE and some of its acyl esters. Though most of these esters induced a decrease of the spontaneous movement immediately after the administration, significant increase of the motor activity in mice could be seen 1 hour after the administration of hexanoate, valerate, butyrate and acetate of DMAE. The effects of propionate and phenylacetate of DMAE have some similarities to that of DMAE.

DMAE and some of its acyl esters increase the spontaneous and exploratory movements in mice, that is, they have the stimulant action on central nervous system according to the proposal by Pfeiffer (4). In the reserpinized mice, the significant recovery of the depressed spontaneous movement could be recognized in both climbing test and hole cross test when acetyl ester of DMAE was given. The administration of DMAE and its propionate led to somewhat increase of the recovery in both the tests.

DMAE and some of its acyl esters can be thought to have the direct action on central nervous system from the fact based on the increase of motor activity in mice after the injection of these drugs, and on the recovery of the depressed spontaneous movement in reserpinized mice.

That acetyl ester of DMAE has more potent action than DMAE, would suggest the possibility that acetylcholine is related to the action of DMAE in the central nervous system as Pfeiffer et al. proposed. However, the conversion of DMAE to acetylcholine does not proceed so rapidly (Groth et al. (9) ) and Ansell et al.(15) suggested that there may be no relationship between the metabolic fate of DMAE a short time after the administration and its pharmacological effect after prolonged dosages.

The action of DMAE induced immediately after the administration can not be ascribed to the conversion of the drug to acetylcholine in the central nervous system. Other possibility may exist, that is, DMAE has a direct effect on the central nervous system as Pepeu et al. (10) suggested. The recovery of the depressed spontaneous movement in reserpinized mice after the administration of these drugs might open the possibility that DMAE affects a certain transmitter in the central nervous system with the exception of acetylcholine. It is
also important to know whether acetyl ester of DMAE reacts like acetylcholine as its proper form or reacts indirectly as DMAE produced after the hydrolysis in the central nervous system.

**SUMMARY**

Smaller changes of spontaneous movement in mice after the single administration of smaller doses of methamphetamine, caffeine, reserpine, and chlorpromazine which can not be found by usual methods for a separate animal, can be detected by the hole cross apparatus.

DMAE and some of its acyl esters have two different actions on the spontaneous movements in mice; depression and excitation can be seen a short time after the single administration. The recovery of the depressed spontaneous movement of the reserpinized mice can be recognized after the single injection of DMAE and its acetate.

**REFERENCES**

1) SIEGEL, P.S. AND HARD, J.S.: *J. gen. Psychol.* 42, 159 (1950)
2) DEWS, P.: *Br. J. Pharmac. Chemother.* 8, 46 (1953)
3) TAKAGI, K., SHIBATA, M., WATANABE, M. AND SAITO, A.: *Yakugaku Zasshi* 87, 873 (1967)
4) PFEIFFER, C.C., JENNY, E.H., GALLAGHER, W., SMITH, R.P., BEVAN, W., KILLAM, K.F., KILLAM, E.K. AND BLACKMORE, W.: *Science, N. Y.* 126, 610 (1957)
5) KÖNIGSMARK, B., KILLAM, E.K. AND KILLAM, K.F.: *J. Pharmac. exp. Ther.* 122, 39A (1958)
6) HIMWICH, H.E.: *A.R.N.M.D.* XXXVII, 356 (1958)
7) BROWN, B.B. AND GANGLOFF, H.: *Fedn. Pro.** 18, 372 (1959)
8) PFEIFFER, C.C. AND JENNY, E.H.: *Ann. N. Y. Acad. Sci.* 66, 755 (1957)
9) GROTH, D.P., BRAIN, T.A. AND PFEIFFER, C.C.: *J. Pharmac. exp. Ther.* 122, 28A (1958)
10) PEPEU, G., FREEDMAN, D.X. AND GIARMAN, N.J.: *J. Pharmac. exp. Ther.* 129, 291 (1960)
11) KIPLINGER, G.F., SWAIN, H.H. AND BRODY, T.M.: *J. Pharmac. exp. Ther.* 122, 37A (1958)
12) SANDBERG, F.: *Arzneimittel-Forsch.* 9, 203 (1959)
13) SCHNEIDER, R. AND TEMES, A.R.: *Br. J. Pharmac. Chemother.* 12, 30 (1957)
14) CHANCE, M.R.A.: *J. Pharmac. exp. Ther.* 87, 214 (1946)
15) ANSELL, G.B. AND SPANNER, S.: *J. Neurochem.* 9, 253 (1962)