Differential Sensitization to Ambulation-Increasing Effect of Methamphetamine after Repeated Administration to Mice in Activity Cages of Different Sizes

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ABSTRACT—We have demonstrated that repeated administration of methamphetamine (MAP) 1–2 mg/kg, s.c. in a tilting-type round activity cage with a diameter of 20 cm (20 cm-cage) at 1–7 day intervals produces progressive enhancement of the ambulation-increasing effect (AIE), showing sensitization (reverse tolerance) in mice. However, almost no sensitization was observed when mice had been pretreated with the same doses of MAP but confined in a narrow environment repeatedly. In this experiment, changes in the sensitization were investigated in mice after repeated pretreatments with MAP at 2 mg/kg at 3–4 day interval for 4 times in activity cages of 5 different sizes (5, 10, 15, 30 and 40 cm in diameter) in comparison with that of in the 20 cm-cage. AIE obtained after MAP in the 5- and 10-cm cages was not enhanced, whereas AIE was significantly enhanced in the cages of more than 15 cm in diameter. There was no significant difference in the enhanced AIE among the 4 groups that received the same pretreatment repeatedly in the cages of 15, 20, 30 and 40 cm in diameter. The present results suggest that in accordance with the all-or-none law, development of the sensitization is affected by the size of the activity cage in which the animals have repeatedly experienced the acute drug effect.

We have demonstrated previously that repeated administration of psychotropic drugs, such as d-amphetamine (1), methamphetamine (2), morphine (3), methylphenidate (4), ephedrine (5), apomorphine (6) and bromocriptine (7), to mice in adequate doses and at adequate intervals produced marked and progressive enhancement of the ambulation-increasing effects (AIE) of the drugs when examined in a tilting-type round activity cage of 20 cm in diameter. This phenomenon, so-called sensitization or reverse tolerance, has been widely supported by many investigators (8–11). In this phenomenon, we often observed poor enhancement when 10 animals were housed together in a relatively small home cage (35(W) × 25(D) × 10(H) cm) (1). In addition, there was almost no development of enhancement in animals that had been pretreated repeatedly with the drugs and confined in a narrow environment (glass jar of 5.5 cm in diameter and 15 cm height) (2–5). These findings suggest that such a sensitization phenomenon is strongly affected by the dimensions of the activity cage in which the animal move about under the drug effect. However, there have been no studies on the systematic relationship between the sensitization and the dimensional factor of the activity cage.

We performed the present studies to investi-
gate changes in the sensitization after repeated pretreatments with methamphetamine in round activity cages of 5 different sizes (diameters ranging from 5 to 40 cm).

MATERIALS AND METHODS

Animals
Adult male mice of the ddY strain weighing 25–30 g at the beginning of the experiment were used. The animals were purchased from Japan Charles River Co. (Atsugi, Japan). The mice were housed in groups of 10 in aluminum cages of the following dimensions, 35(W) X 25(D) X 10(H) cm, with wooden-flake bedding and given free access to a solid diet, MF (Oriental Yeast Co., Tokyo) and tap water except during the experiments. The breeding room was artificially illuminated by fluorescent lamps with a 12 hr light-dark cycle (light on 6:00–18:00), and the room temperature was controlled to 23 ± 2°C.

Measurement of ambulatory activity
The ambulatory activity of the mice was measured by the tilting cage method (AMB-M20, Ohara and Co., Ltd., Tokyo). The principle of the device and method were described in detail by Hirabayashi et al. (12). Briefly, each slight tilt of the plexiglass basin-type round activity cage (20 cm in diameter and 18 cm height) according to a horizontal movement over 5–10 cm of the mouse was detected by 3 micro-switches fixed to the cage box. Each mouse was placed in the activity cage (regular activity cage; R-cage), and the ambulatory activity counts were recorded every 10 min for 30 min before and for 180 min after drug administration. The activity was usually measured at 10:00–15:00.

Drugs
Methamphetamine hydrochloride (MAP, Philopon, Dainippon Pharmaceutical Co., Ltd., Osaka) and physiological saline (saline) were used. MAP was used in the salt form and dissolved in purified water. It was administered subcutaneously, and the volume administered was fixed to 0.1 ml per 10 g body weight of a mouse.

Different sizes of activity cage
Five different sizes of plexiglass basin-type round activity cages without a tilting work device were used, with the exception of the R-cage. They were 5 cm (5 cm-cage), 10 cm (10 cm-cage), 15 cm (15 cm-cage), 30 cm (30 cm-cage) or 40 cm (40 cm-cage) in diameter and all 30 cm in height.

Drug administration schedules
As we have reported in the previous papers (2, 10), there is an optimum dose and administration interval necessary to observe a clear sensitization to AIE of MAP in the R-cage. The dose of 2 mg/kg s.c. and 3–7 day intervals are considered to be optimum in mice. Thus, the following experiments were conducted as shown in Table 1.

Two hundred forty mice were divided into 6 groups (Groups I–VI) of 40 animals. The first group (Group I) received MAP at 2 mg/kg at intervals of 3–4 days, 5 times; and their activities were measured for 180 min after each administration.

Half the mice of Groups II, III, IV, V and VI received repeated pretreatments with either MAP at 2 mg/kg or saline at intervals of 3–4 days, 4 times, in a 5 cm-, 10 cm-, 15 cm-, 30 cm- and 40 cm-cage, respectively. Three days after the final pretreatment, all mice received MAP at 2 mg/kg as the test dose, and their ambulatory activities were measured for 180 min in the R-cages.

Statistical evaluation
Significant differences of mean activity counts were evaluated by Student’s t-test and 2-way analysis of variance.

RESULTS

Development of the sensitization after repeated administration of MAP in the regular activity cage
Figure 1 shows time-course changes in mean
Table 1. Experimental conditions

| Groups | Drug administration       | Activity cage size (diameter) | Doses of methamphetamine | No. of animals |
|--------|---------------------------|-------------------------------|---------------------------|----------------|
| I      | Treatments in regular activity cage | 20 cm                         | 2 mg/kg × 5               | 40             |
| II     | Pretreatments in cage     | 5 cm                          | 2 mg/kg × 4, saline × 4   | 20             |
| III    | Pretreatments in cage     | 10 cm                         | 2 mg/kg × 4, saline × 4   | 20             |
| IV     | Pretreatments in cage     | 15 cm                         | 2 mg/kg × 4, saline × 4   | 20             |
| V      | Pretreatments in cage     | 30 cm                         | 2 mg/kg × 4, saline × 4   | 20             |
| VI     | Pretreatments in cage     | 40 cm                         | 2 mg/kg × 4, saline × 4   | 20             |

Fig. 1. Time-course changes in mean ambulatory activity counts of mice for 180 min after repeated administration of 2 mg/kg MAP at intervals of 3–4 days, 5 times, in the R-cage. The abscissa denotes the time after MAP and the ordinate, the mean activity counts per 10 min. Significant difference (2-way analysis of variance, activity × time interaction): 1st vs. 2nd (P < 0.001, F = 15.8), 1st vs. 3rd (P < 0.0001, F = 41.4), 1st vs. 4th (P < 0.0001, F = 61.8), 1st vs. 5th (P < 0.0001, F = 56.8).
ambulatory activity counts on the same coordinate for 180 min after repeated administration of MAP at 2 mg/kg at intervals of 3–4 days, 5 times. The activity was increased after the drug with a maximum at 30–40 min. Then the AIE was progressively enhanced according to the number of repetitions, and peak values were markedly elevated and durations of the effects were also prolonged after the 2nd administration and thereafter. The activity counts after the 2nd and later administrations were significantly different from that in the 1st administration, showing a marked sensitization phenomenon.

Changes in the sensitization after repeated pretreatments with MAP in 5 different sizes of activity cage

Figure 2 shows the results of the test dose of 2 mg/kg MAP in the R-cage after repeated pretreatments with either MAP or saline in 5 different sizes of activity cage. The activity patterns of Groups II and III which had been repeatedly pretreated with MAP in the 5 cm- and 10 cm-cage did not differ from those of the saline-control groups. In contrast, Groups IV, V and VI which had been repeatedly pretreated with the same dose of MAP in the 15 cm-, 30 cm- and 40 cm-cage, respectively, showed markedly higher activity patterns (i.e., development of the sensitization) than those of Groups II and III.

Comparisons of the development of sensitization obtained in 5 different sizes of activity cage and in the regular activity cage

Figure 3 shows comparisons of mean overall activity counts during a 180 min measuring period obtained by the test dose in Groups II–VI and the control group, and those of the 1st

![Graph](image-url)
and the 5th drug administration in Group I. In addition, the activity counts of the 5 saline-control groups did not differ statistically from each other, and therefore they were pooled as one control group. There was no difference in the counts among Group II, Group III, the control group and the 1st administration in Group I. Similarly, there was no difference in the counts among Groups IV-VI and the 5th administration in Group I, but they showed higher activity levels than those of the preceding groups. Thus, a significant difference in the development of sensitization between the former and the latter groups was confirmed.

**DISCUSSION**

The present experiment demonstrated clearly that the size of the activity cage differentially influences the sensitization to AIE of MAP. AIE of MAP in mice that received the pretreatments with the same drug in the 5- and 10-cm cages was not enhanced. In contrast, AIE was significantly enhanced when the mice were similarly pretreated in the cages of more than 15 cm in diameter. There was no significant difference in the enhanced AIE among the 4 groups that received the same pretreatments in the 15-, 20-, 30- and 40-cm cages, respectively. These results suggest that in accordance with the all-or-none law, development of the sensitization is affected by the size of the activity cage in which the animal has repeatedly experienced the acute drug effect.

We have already indicated a possible conditioning mechanism of AIE sensitization after repeated administration of several psychotropic drugs in the R-cage and attenuation of the sensitization in narrow-dimensional environment (1-7). Other investigators (13-18) also pointed out an important role of distinctive environment on the conditioned drug effects, but no dimensional factor of the activity cage.

On the other hand, Kuczenski and Leith
(19) and Beninger and Hahn (20) reported that the sensitization after d-amphetamine was blocked by neuroleptics and was dependent upon the activity of the dopaminergic neurons in the CNS. It is interesting to note that Hayashi et al. in our laboratory (21) showed no change in brain catecholamine contents and the receptor binding sites after rats were pretreated with MAP in a narrow environment. Kilbey and Sannerud (9) have explained that lack of the sensitization (19, 20) after chronic combined administration of neuroleptics with d-amphetamine is not due to a block of dopamine receptors, but rather due to a block of the drug-induced hyperactivity associated with the environmental stimuli.

Finally, it has been known that drug abusers tend to switch from a given drug to another, and their drug seeking behaviors may be conditioned partially to the environmental or social contexts (22, 23). The present findings may support such observations in relation to environmental effects.

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