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Enhancing Effect of Methamphetamine on Ambulatory Activity Produced by Repeated Administration in Mice

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HIRABAYASHI, M. AND M. R. ALAM. *Enhancing effect of methamphetamine on ambulatory activity produced by repeated administration in mice.* PHARMAC. BIOCHEM. BEHAV. 15(6) 925–932, 1981.—Effect of repeated administration of methamphetamine (MAM) on ambulatory activity was studied under various experimental conditions. Nine groups of mice received 10 doses of 1, 2 or 4 mg/kg MAM on a daily, 3–4 day or weekly schedule. Increases in activity were enhanced progressively in proportion to the number of MAM doses, but the extent varied with the dose and interval of repetition. One mg/kg caused slight enhancement under all conditions. In the case of 2 mg/kg, marked enhancement was observed until the 7th–8th administration in 3–7 day schedules, but poorer enhancement was elicited by daily administration. Four mg/kg of daily repetition caused no enhancement, because of marked stereotyped behavior which competed with ambulation. However, the enhancement was observed in the 3–7 day schedule when stereotyped behavior was not prominent. The enhancement was well maintained after a 2 months drug-free period. When a mouse was confined in a jar of small diameter to impede ambulation after MAM, the enhancement was blocked. These results suggest a possibility that learning of drug effect in association with environmental factors may play an important role in the enhancing effect.

Repeated methamphetamine Ambulatory activity Enhancing effect Learning of drug effect Mice

MANY drugs used in clinical practice are administered for prolonged periods. However, it is well known that drug effects are sometimes modified by repeated administration, i.e., development of tolerance or reverse tolerance [16,24]. We already have observed that when 2.5 mg/kg of d-amphetamine was given at 1–7 day intervals, motor activity was increased progressively with each administration. Similar behavioral changes were produced after 10 mg/kg of morphine or 20 mg/kg of cocaine [5, 22, 23]. We demonstrated that such effects were strongly influenced by environmental situations in association with drug experiences of the experiment. Hayashi *et al.* [4] in our laboratory have reported recently that such a phenomenon may be due to conditioned drug effects.

The purpose of this paper is to investigate the behavioral properties of the enhancing effect produced by the repeated administration of methamphetamine.

METHOD

Animals

Male dd-strain mice, aged 21 days, were supplied after weaning from the breeding colony of Gunma University, Medical School. They were divided into 9 groups of 8–9 animals and housed in aluminum cages, 32 (W) × 25 (D) × 10 (H) cm, and solid diet MF (Oriental Yeast Co., Tokyo) and tap water were given freely. After a month, 561 animals weighing 25–30 g were used in the experiments. Throughout the periods of the experiments, each group of mice was kept in its respective cage except during the times of drug administration and measurement of ambulatory activity.

Drugs

Methamphetamine hydrochloride (MAM) 1, 2, 4 and 8 mg/kg and physiological saline (saline) were given SC. MAM was used in salt form, and was dissolved in purified water. The solution was appropriately diluted in volumes of 0.05–0.1 ml per 10 g of body weight.

Determination of Ambulatory Activity

The ambulatory activity of each mouse was determined by the tilting cage method, devised by Tadokoro and Takano [21] in our laboratory. The principle of the method is as follows; each mouse is placed in an open round cage 25 cm in diameter. Any slight tilt of the activity cage caused by ambulatory movement in excess of 5–10 cm is detected by a microswitch which activates an electromagnetic counter. The method has already been reported in detail by us [6,7].

Before the drug administration, the activity counts were recorded for 30 min in 10 min intervals after placing the mouse in the activity cage. Then, the drug or saline was

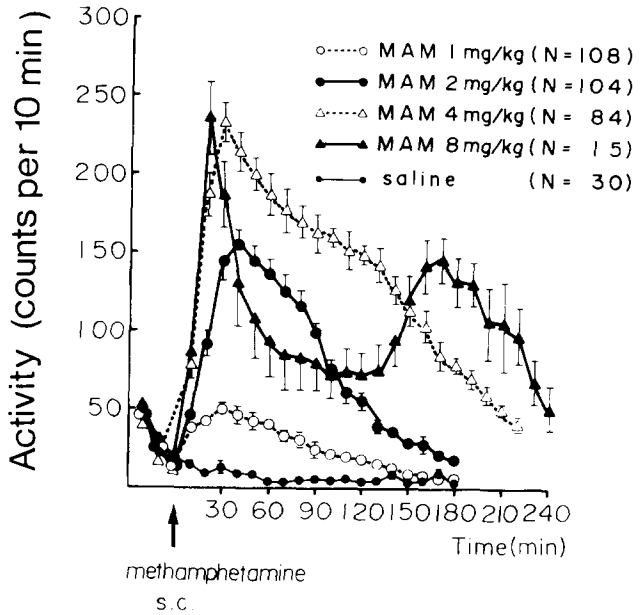

FIG. 1. Temporal changes in the ambulation-increasing effect induced by MAM 1–8 mg/kg and saline in mice. Each point represents mean counts ± SE for a 10 min period. The abscissa denotes times after MAM administration. MAM 1–4 mg/kg increased ambulatory activity in a dose-dependent manner, showing a high peak and prolonged duration, but 8 mg/kg produced a biphasic pattern.

given and the number of counts in every 10 min period was subsequently recorded for 180–240 min. Administration was repeated 10 times at intervals of 1, 3–4 and 7 days in the activity cage and 5 times in the glass jar. The measurements were carried out between 10:00 a.m. and 3:00 p.m., using 15–17 activity cages of the same type. Room temperature was maintained at 20–25°C. Furthermore, activity was observed in the same individually-assigned cage throughout the measurement periods. All experiments were conducted under normal laboratory illumination with 60 W fluorescent strip lights (approximately 100 Lux). Statistical significance of the difference between mean values of the measurements was determined by the Student's *t*-test. The other specific conditions will be given in the Results section.

RESULTS

Ambulation-Increasing Effect Following the Initial MAM Administration

Five groups of mice, each numbering 15–108 animals, were given 1, 2, 4 and 8 mg/kg of MAM or saline, and the temporal changes in ambulation were observed. Figure 1 represents the activity patterns obtained by plotting the mean counts of the 5 groups on the same coordinate. The abscissa denotes time and the ordinate the mean counts for the 10 min periods with standard errors.

Saline administration did not produce a change, whereas MAM 1–4 mg/kg increased ambulatory activity in a dose-dependent manner, showing a high peak and prolonged duration. However, a dose of 8 mg/kg exhibited a biphasic pattern with peaks at 20 and 170 min. The activity decline following the first peak was associated with an increase in stereotyped behaviors observed in forms of rearing, continuous sniffing, head twitching or circular movement, which competed with ambulatory activity. The stereotyped behaviors were often mixed with ambulation, and as the stereotyped behaviors attenuated, the second increase in the activity was observed.

General Symptoms of Mice Throughout the Repeated Administration of MAM

The animals gradually became habituated to handling for injections, and the activity level prior to drug administration tended to be lower with successive repetitions of experiment. However, when the mice had received 2 or 4 mg/kg of MAM repeatedly, they sometimes exhibited restlessness and aggression such as inflicting damage on each other by biting the tail, rump or back, after replacement in their home cages. However, no body weight loss due to the drug administration was observed even in the daily administration.

Enhancement of Ambulation-Increasing Effect Produced by Repeated Administration of MAM

Repeated administration of saline did not produce any change in activity. Figure 2 represents the ambulation patterns obtained by the first, 3rd, 5th, 7th and 10th administration of MAM 1 mg/kg on the same coordinate. The patterns of the 2nd, 4th, 6th, 8th and 9th administration were omitted to simplify the figure. The figures near each curve denote the ordinal numbers of administration. As seen in Fig. 2, the ambulation-increasing effect of MAM was progressively enhanced with each administration, though slightly. Moreover, the time required to reach the peak become shorter and the duration of the effect was prolonged. The maximum enhancement was observed within 7–8 times of repetition regardless of administration interval, and each extent was almost the same. The extent of maximum enhancement exceeded that of the initial administration of MAM 2 mg/kg as shown in Fig. 1.

Figure 3 represents the ambulation patterns obtained by the first, 3rd, 5th, 7th and 10th administration of MAM 2 mg/kg on the same coordinate. The progressive enhancement of the effect was observed more clearly than in the case of 1 mg/kg, but the extent varied according to the interval between administrations. The maximum enhancement was observed within 4–5 times of daily repetition, whereas in the case of every 3–4 or 7 days repetition, the enhancement progressed until the 7th–8th times of repetition, concurrent with a prolonged duration of the effect. The extent of the maximum enhancement in this case exceeded that of the initial administration of 4 mg/kg as shown in Fig. 1.

Figure 4 represents the results of repeated administration of MAM 4 mg/kg graphed in the same way as in Figs. 2 and 3. Comparison with the results in 1 mg/kg- and 2 mg/kg-given cases revealed marked differences in the change of ambulation-increasing patterns. Following the daily administration, marked stereotyped behavior developed by the 3rd administration, and it was enhanced thereafter, exhibiting multiphasic activity patterns. In the case of every 3–4 days repetition, stereotyped behavior was also interspersed among the ambulatory movement in the 2nd and later administrations, though the extent was of a lower level than that in the daily repetition. A biphasic pattern was observed with peaks at 30 and 120 min. The transient decline in activity after the first peak was associated with an increase in stereotyped behavior. As the stereotypy attenuated, a second peak in activity was observed. The second peak was progressively enhanced and the duration of the effect was prolonged following each repetition. However, when the administration

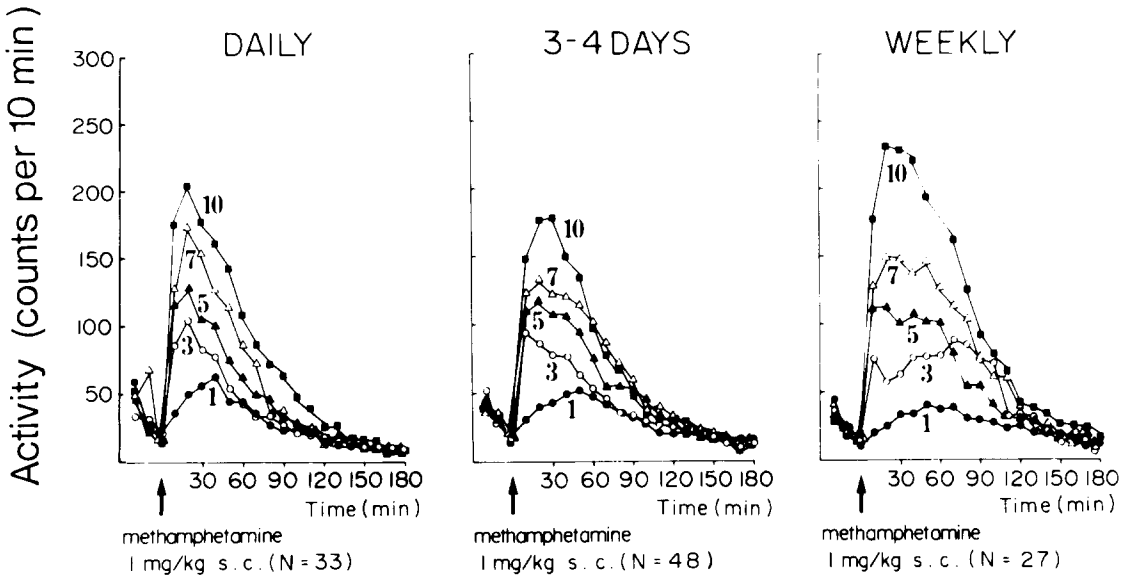

FIG. 2. Ambulation-increasing patterns obtained by the first, 3rd, 5th, 7th and 10th administration of MAM 1 mg/kg at intervals of 1, 3–4 and 7 days. The patterns obtained by the 2nd, 4th, 6th, 8th and 9th are omitted to simplify the figure. The figures near each curve denote ordinal numbers of administration. The increasing effect was progressively enhanced with each administration, though slightly. The extent of the maximum enhancement was almost the same regardless of administration interval. Left panel: Daily treated group. Central panel: Every 3–4 days treated group. Right panel: Weekly treated group.

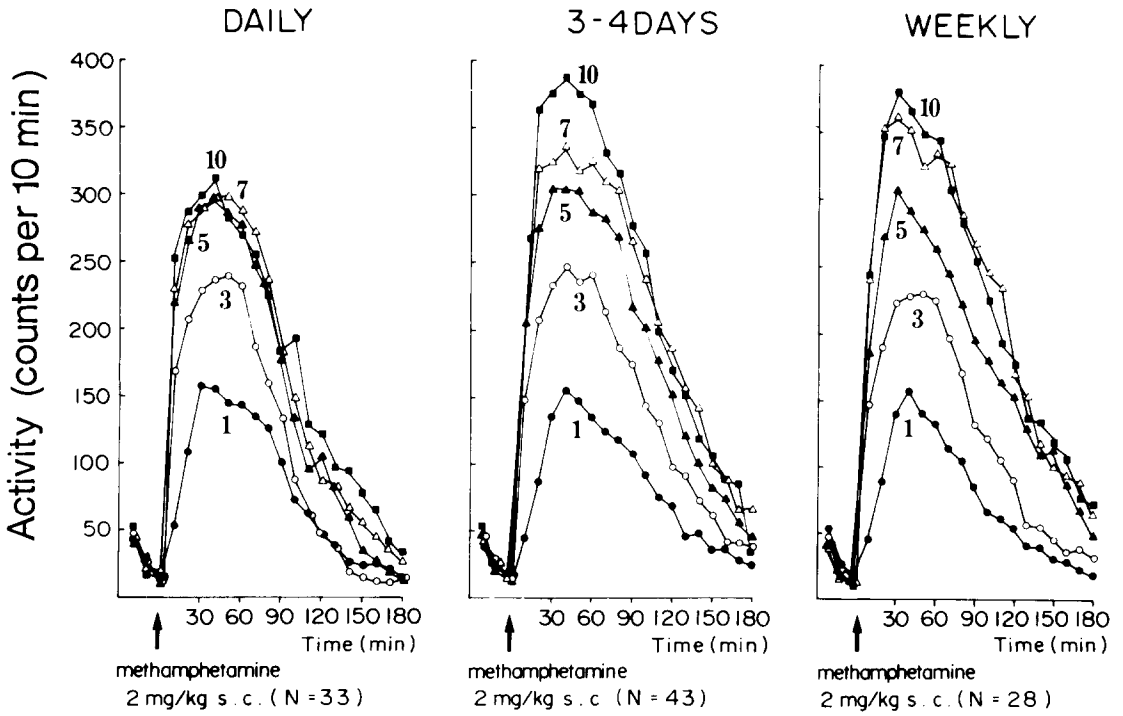

FIG. 3. Ambulation-increasing patterns obtained by the first, 3rd, 5th, 7th and 10th administration of MAM 2 mg/kg as expressed in Fig. 2. The progressive enhancement was observed more clearly than in the case of 1 mg/kg. The enhancement was more evidently developed when the administration interval was 3–7 days than in the case of daily administration.

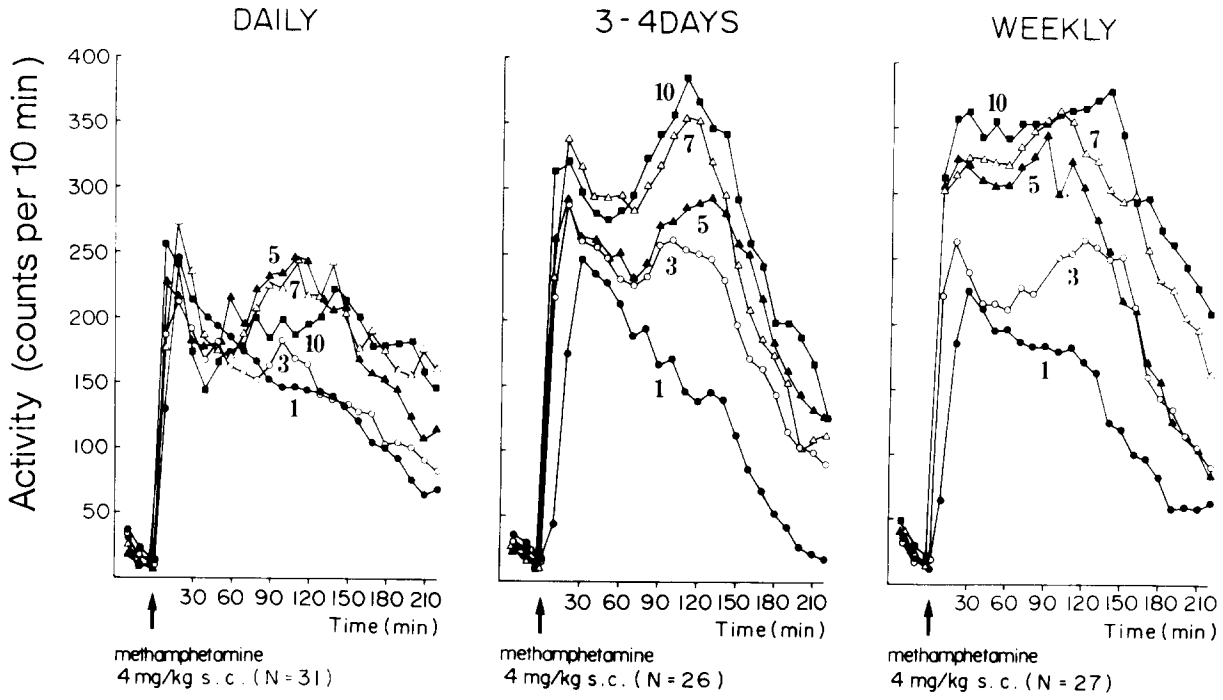

FIG. 4. Ambulation-increasing patterns obtained by the first, 3rd, 5th, 7th and 10th administration of MAM 4 mg/kg as expressed in Figs. 2 and 3. The enhancement was most prominent in the 7 days-repetition group.

was repeated every 7 days, the development of the stereotyped behavior was milder than that in the case of every 3–4 days, and consequently the ambulation-increasing patterns exhibited a peak with a flat curve.

When the total activity counts for 180 min after the drug in each case were compared between the first and 2nd–10th administrations, significant increases were always observed after the 2nd–4th and later administrations ($p<0.05$) of 1 mg/kg in each interval, and after the 3rd and later administrations both of 2 and 4 mg/kg ($p<0.01$) at intervals of 3–4 and 7 days as shown in Fig. 5.

Persistence of Enhancing Effect

After 10 repetitions, MAM was withdrawn for 2 months. The ambulation-increasing effect was then examined in the 11th administration. Figure 6 represents the comparison of changes among the first and the 10th administration of MAM 2 and 4 mg/kg at the interval of 3–4 days, and the 11th after 2 months. Repetition with saline did not produce significant change, and the 11th administration was not done. The ambulation-increasing pattern at the 11th administration corresponded to that after 7–8 repetitions with 2 mg/kg of MAM, and to that after 4–5 repetitions with 4 mg/kg. There was no significant difference between the activities of the 10th and the 11th administration except at a 30–50 min period after 2 mg/kg and at a 90–100 min period after 4 mg/kg ($p<0.05$). However, significant differences were observed in the activities between the first and the 11th administration throughout the time course after 2 mg/kg and at a 90–220 min period after 4 mg/kg ($p<0.01$–0.001). Moreover, the stereotyped behavior was reproduced even after 2 months of withdrawal, and the biphasic pattern was observed as irreversible.

Ambulation-Increasing Effect of MAM after Repeated Motor-Impeding Pretreatments

The repeated performance of actual movements under drug effect (learning of the effect) may be necessary for the enhancing effect. Therefore, the following experiment was conducted. The six groups of mice were subdivided into groups of two for drug and saline pretreatments. Three subgroups were given 2 mg/kg and the other three 4 mg/kg of MAM, while the other 6 groups received saline, at intervals of 1, 3–4 and 7 days for 5 times. All groups of mice were individually confined in a glass jar (6 cm in diameter, 15 cm high) for 180 min to impede ambulatory movement. In the 6th administration, all mice were given 2 or 4 mg/kg of MAM. Then, they were placed in the activity cages and allowed to move freely. Figures 7 and 8 represent the results. As seen herein, there was no significant difference in the activity between MAM- and saline-pretreated groups. Moreover, the ambulation patterns in these experiments were similar to those following the initial MAM administration as shown in Fig. 1, and clearly different from those after the 6th administration as shown in Figs. 3 and 4. The main movement in the jars-confined animals was rearing, but the rearing was maintained temporarily after they were placed in the activity cage.

DISCUSSION

Drug actions are generally multifarious, and behaviorally may not produce the same effect on repeated administration. Tolerance develops to the anorexigenic and peripheral-sympathomimetic effects to amphetamines when give repeatedly [18]. However, there have been many reports concerning the progressive enhancement of the central-stimulating effects of d-amphetamine following repeated

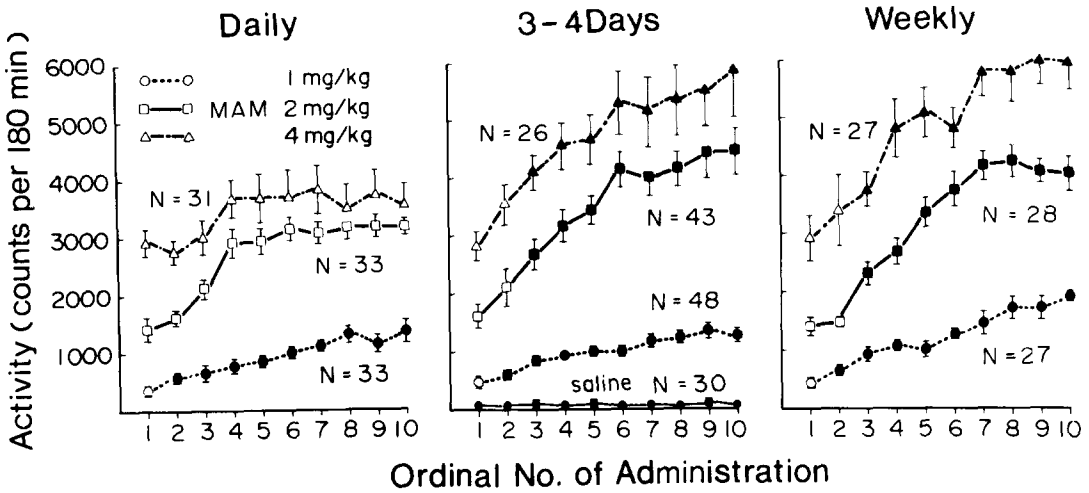

FIG. 5. Comparisons of the total activity for 180 min among the first–10th administration of the nine conditions shown. The enhancement developed in proportion to the number of administration under any condition except in case of daily repetition of 2 and 4 mg/kg. ●, ■ and ▲ are significantly different from the activity of the first administration ($p<0.05$, $p<0.01$ and $p<0.01$).

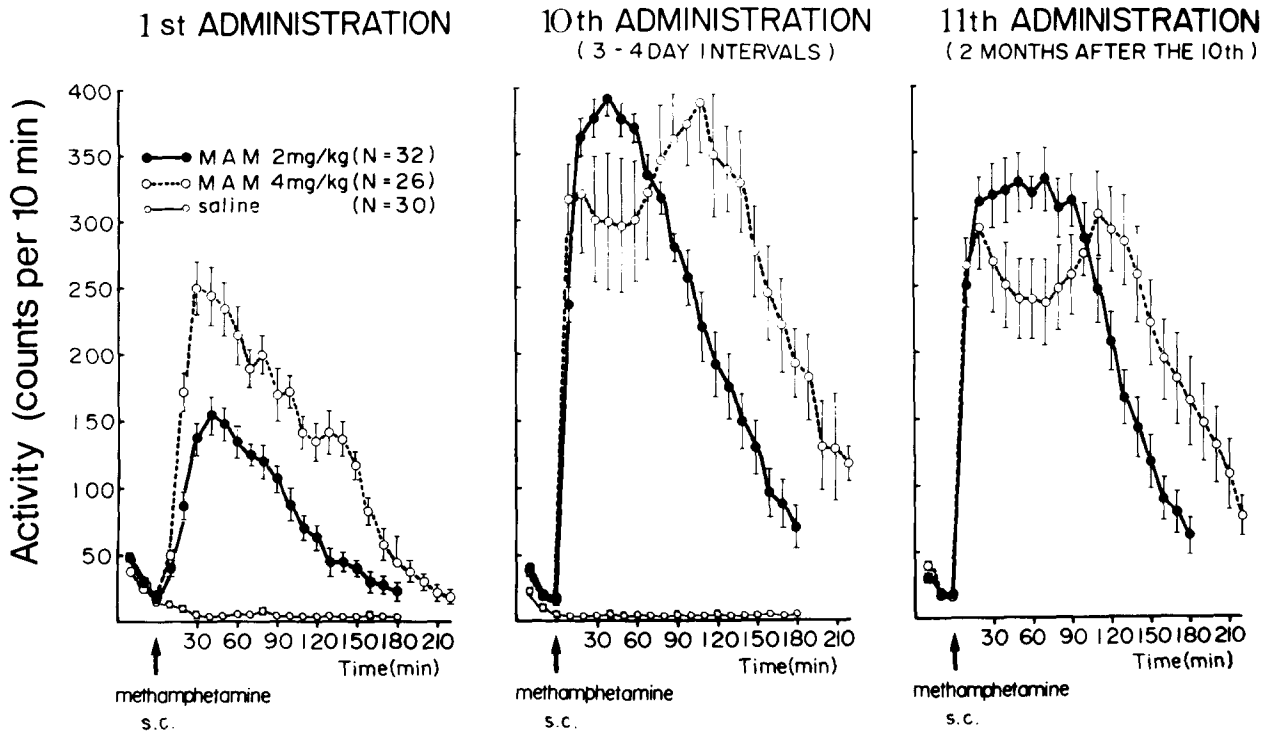

FIG. 6. Comparisons of changes in the ambulation-increasing effect on a 3–4 day schedule among the first, the 10th administration of MAM 2 and 4 mg/kg, and the 11th after 2 months of withdrawal. The enhancement, once established, persisted for a long time and became apparently irreversible.

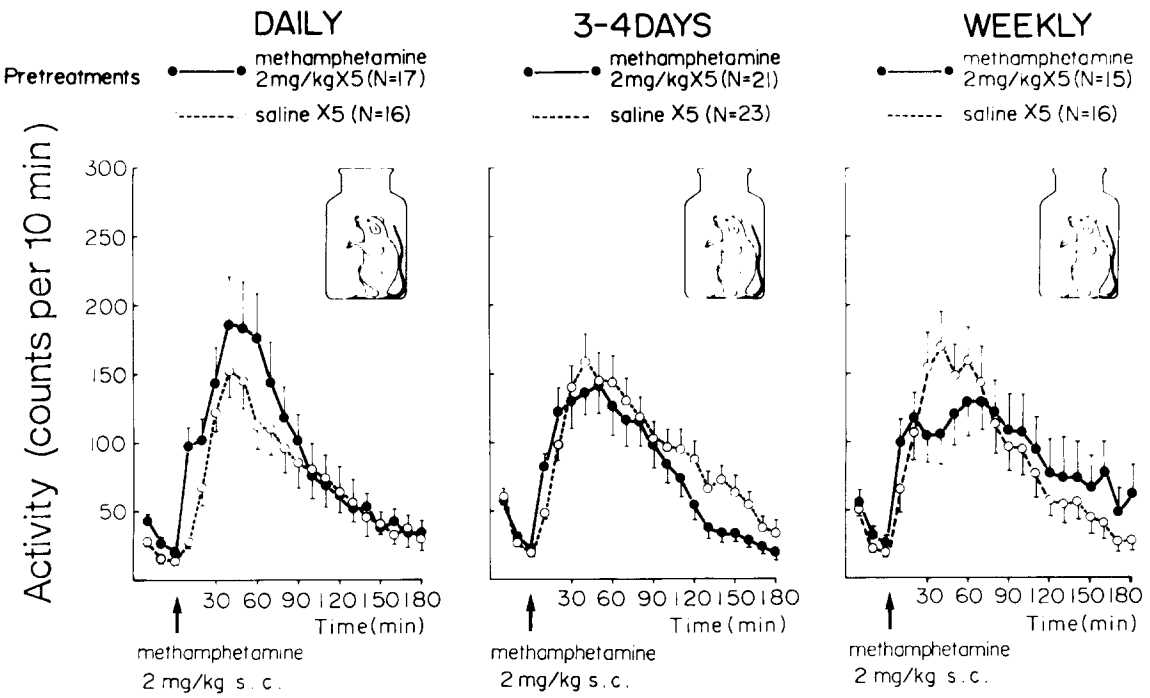

FIG. 7. Ambulation-increasing patterns after MAM 2 mg/kg following 5 pretreatments with MAM 2 mg/kg or saline at intervals of 1, 3–4 and 7 days in a jar. No marked difference in the activity was observed between MAM- and saline-pretreated groups.

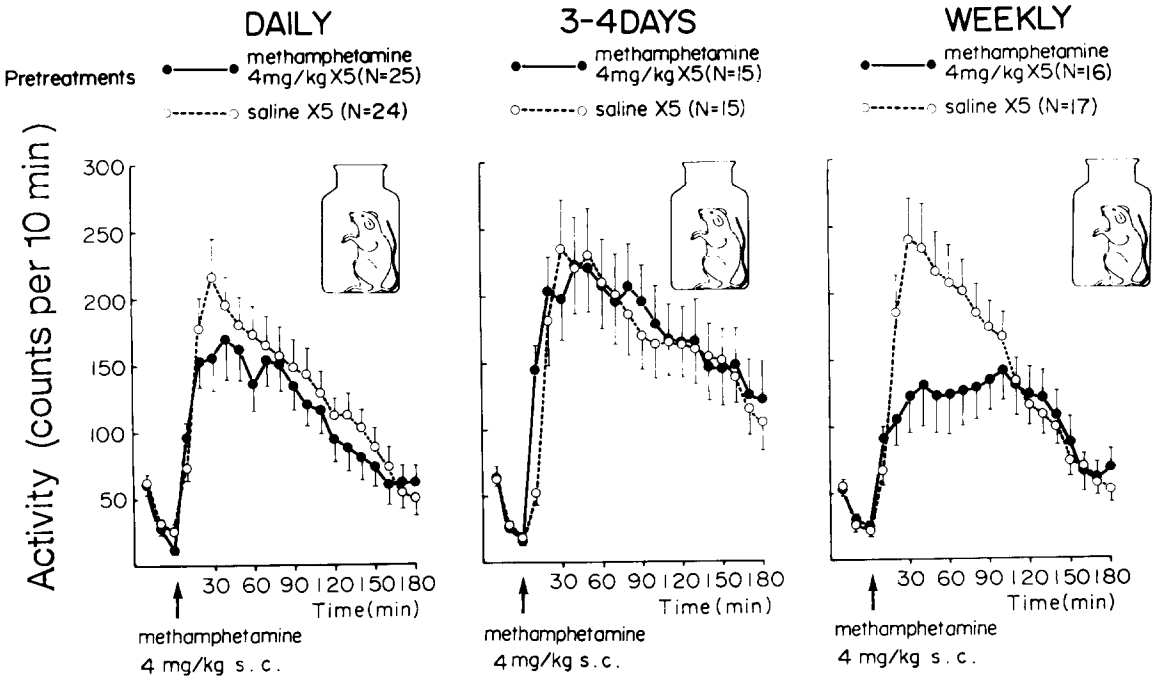

FIG. 8. Ambulation-increasing patterns after MAM 4 mg/kg following 5 pretreatments with MAM 4 mg/kg or saline at intervals of 1, 3–4 and 7 days in a jar. No marked difference in the activity was observed between MAM- and saline-pretreated groups.

administration [10, 13, 14, 18, 24]. The mechanism of such enhancement is still to be established.

The results presented here showed that the repeated administration of MAM produced the progressive enhancement of effects with respect to ambulation. Moreover, the stereotyped behavior displayed a relationship competitive to the ambulatory activity, and was produced when a larger dose was given at shorter intervals. The extent of the enhancement in terms of changes in the ambulatory activity may be controlled by varying the combinations of dosage and the interval of repetition. In a dose of 1 mg/kg, enhancement was observed in the daily administration, but in a dose of 4 mg/kg, the longer the administration interval, the more prominent was the enhancement. Browne and Segal [2] have reported that a metabolite of d-amphetamine in the rat brain is not detectable after six days, at which time behavioral augmentation can be demonstrated. These results suggest that the enhancement produced by intermittent treatment may not result from an accumulation of the drug. The enhancement, once established, was reproducible even after long discontinuation of the drug, and was considered to be almost irreversible. It has been reported that the similar enhancement produced by d-amphetamine or cocaine persists for a long period [18,19].

The relationship between drug experience and environmental factors constitutes the most characteristic trait of the present results. It was confirmed that the enhancement of ambulatory activity did not develop without sufficient space for free movement even when the drug was given repeatedly in adequate doses at adequate intervals. Furthermore, our laboratory demonstrated that 2.5 mg/kg of d-amphetamine failed to produce the enhancement after repeated pretreatments in combination with a sufficient dose of chlorpromazine [8]. We have also reported that the enhancement developed in mice when the floor space of the round activity cage was over 16 cm in diameter [12]. An intimate relationship between pharmacological actions and experimental situations was pointed out by many researchers [13, 14, 25]. Kuribara and Tadokoro [11] in our laboratory confirmed that the attenuating effect of diazepam on conditioned suppression in rats was markedly enhanced by repeated administration, regardless of the interval. Recently Hayashi and Tadokoro [3] have observed that the repeated administration of chlorpromazine or haloperidol produced an enhancement of suppressive effect on conditioned avoidance response in rats, whereas tolerance conversely developed to suppressive effect by diazepam on avoidance response. Furthermore, they emphasize a possibility that the enhancement and tolerance in these cases may have resulted from the learning of the drug effect which is correlated closely to the experimental situations. The enhancement observed in the present experiments is also considered to be due to the learning of drug effect.

On the other hand, amphetamines are thought to act through the stimulation of release or the inhibition of reuptake and metabolism of catecholamines (CA) at the synaptic sites in the brain [18]. Some reports explained that the enhancement seems to be related to the norepinephrine and dopamine contents in the brain [2,17]. These reports were based on a marked decrease in the CA contents produced by repeated administration of larger doses of d-amphetamine which caused marked stereotyped behavior after administration [2,17]. Some reports also explained that the progressive enhancement of stereotyped behavior is due to the increase in the brain dopamine release or to the supersensitivity of the dopaminergic receptor which is elicited by the repeated administration of MAM [9,15]. However, such interpretation may not account for our results, i.e., the enhancing effect in terms of the ambulatory activity.

Brain CA levels decreased by the pretreatments of reserpine or alpha-methyl-para-tyrosine are considered to produce potentiation or reduction of locomotor activity to d-amphetamine [1,20]. These results suggest that there may be a relationship between MAM-induced enhancement and changes in brain CA levels. Therefore, a role of the brain CA contents may not be ignored in the enhancement phenomenon. Systematic neurochemical research connected with careful behavioral observations are required to solve such problems.

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