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Schedule Induced Self-Injection of D-Amphetamine by Naive Animals

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TAKAHASHI, R. N., G. SINGER AND T. P. S. OEI. *Schedule induced self-injection of d-amphetamine by naive animals.* PHARMAC. BIOCHEM. BEHAV. 9(6) 857–861, 1978.—Naive rats were initially allowed to self-inject 0.05, 0.2 or 0.8 mg/kg infusion of d-amphetamine under conditions of 100% body weight and 80% reduced body weight with or without a fixed time-1 min (FT-1) food delivery schedule. In a further experiment using 0.05 mg/kg amphetamine the self-injection rates of animals under 100% and 80% body weight were compared with rates of animals at 90% body weight with or without FT-1 schedule conditions. The results showed that (a) when body weight is reduced to 80% animals without an FT-1 food delivery schedule self-inject at a higher rate than those on the FT-1 schedule, which is contrary to self-injection patterns found for nicotine, methadone and heroin, (b) the lowest dose of 0.05 mg/kg produced the highest self-injection rate in all conditions, and (c) the highest level of food deprivation (80% body weight) is more effective than normal and 90% body weight in producing amphetamine self-injection. These findings indicate that to initiate high levels of amphetamine self-injection an interaction of food deprivation state and the pharmacological property of the drug is a critical variable.

Self-injection Schedule condition d-Amphetamine

IN A RECENT series of experiments [5,6] a new method for inducing voluntary intake of large quantities of drugs was developed. This method which involves a combination of self-injection procedure and scheduled food delivery permits the study of the interaction of environmental, nutritional and pharmacological factors. Recent findings using this method have shown that rats increase their rate of self-injection of nicotine, methadone or heroin compared to saline under reduced body weight conditions on a fixed time-1 min (FT-1) food delivery schedule [5,6]. The authors suggested that the findings were due to the interaction of pharmacological and environmental factors which maximized drug self-injection.

There have been many reports of self-administration of both isomers of amphetamine by rats [7,16], dogs [9] and monkeys [2] with chronic jugular catheters where every lever-pressing response produced an intravenous drug injection. However, in most of these studies the interaction of pharmacological, environmental and nutritional factors has been neglected. Since the initiation and the maintenance of a greater rate of self-injection is important to assess a dependence potential, the purpose of the present experiments was to examine in the first instance the self-injection pattern of three doses of d-amphetamine under this new paradigm [6]. In a further experiment an optimum dose of amphetamine was selected and the same basic design was used to examine the influence of body weight reduction levels on self-injection rate.

METHOD

Animals

Eighty-five naive male Wistar albino rats weighing approximately 400 g were used. All animals were housed individually in a temperature controlled room with a 12 hr light/dark cycle. Food and water were available ad lib. In experiments requiring rats at 80% of their body weight these were reduced prior to surgery and then maintained at that weight, with water available ad lib.

Apparatus

The experimental chamber was a modified operant box (35×32×32 cm) with a bar and food pellet dispensing unit attached to one side of the walls. The bar was situated 5 cm and the pellet dispensing unit 3 cm from the grid floor. The bar operated a syringe infusion pump (Sage Instruments, model 341) which delivered 0.07 ml of d-amphetamine solution or saline when triggered. A timing device set for a fixed interval of 5 sec was incorporated into the drug delivery system so that any further bar presses by the animals during the 5 sec infusions were not rewarded with drug injections. Cumulative records were used to record the number of bar presses and infusions during test sessions. Noyes food pellets (45 mg) were delivered regularly, one each minute, to the animal when the fixed time-1 min (FT-1) schedule was operating.

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Drugs

Solutions of d-amphetamine sulphate (Sigma Chemical Co.) were prepared for intravenous administration prior to each test session by dissolving it in 0.9% sterile saline. The anaesthetic used for the surgery consisted of a combination of pentobarbital sodium and the chloral hydrate and the solution was injected intraperitoneally.

GENERAL PROCEDURE

All animals were surgically implanted with a jugular cannula under anaesthesia. Cannulae of SP28 polythylene tubing were maintained in position by leather jackets worn by each animal. The catheter was connected to a flexible swivel system allowing each animal unrestricted movement. Animals were allowed three days to recover from surgery before being assigned to an experimental group.

Following recovery the animals were placed in the operant box for 1 hr/day testing sessions for 6 consecutive days at the same time each day. Each experiment commenced by priming the animal with an initial dose of drug solution or saline.

EXPERIMENT 1
DOSE-RESPONSE PATTERN OF D-AMPHETAMINE SELF-INJECTION AT 80%, 80%+FT-1 MIN AND 100% BODY WEIGHT

In this experiment, the dose-response relationship of amphetamine under the three conditions was determined in an attempt to establish the optimal condition for a higher intake of amphetamine.

Animals

Seventy rats were placed in the experimental chamber with saline or d-amphetamine at 0.05, 0.2 and 0.8 mg/kg/infusion available through bar pressings.

Procedure

For each drug condition the animals were randomly allocated to the three schedule conditions, 100% body weight, 80% body weight under an FT-1 schedule and 80% body weight without a schedule. The animals were weighed before each daily session. The gross behaviour during the experimental session was observed and recorded throughout 6 days. Daily record of water intake at the home cage was also monitored.

RESULTS

The overall means of infusion/hour/day for the three groups self-injecting d-amphetamine at each dose and saline is shown in Fig. 1.

A two-way Analysis of Variance (ANOVA) of these data showed significant main effects of drug treatments, $F(3,58)=3.966$, $p<0.025$, and schedule conditions, $F(2,58)=6.578$, $p<0.01$. However, the interaction between drug treatments and schedule conditions was not significant, $F(6,58)=2.151$, $p>0.05$. These data suggest that rats under different schedule conditions and different dose levels self-injected significantly different amounts of d-amphetamine. Inspection of Fig. 1 shows that 80% reduced body weight animals without an FT-1 schedule self-injected consistently more amphetamine at 0.05, 0.2 and 0.8 mg/kg when compared to animals at 80% body weight with FT-1 and 100% body weight. A Scheffé post hoc test showed that at dose

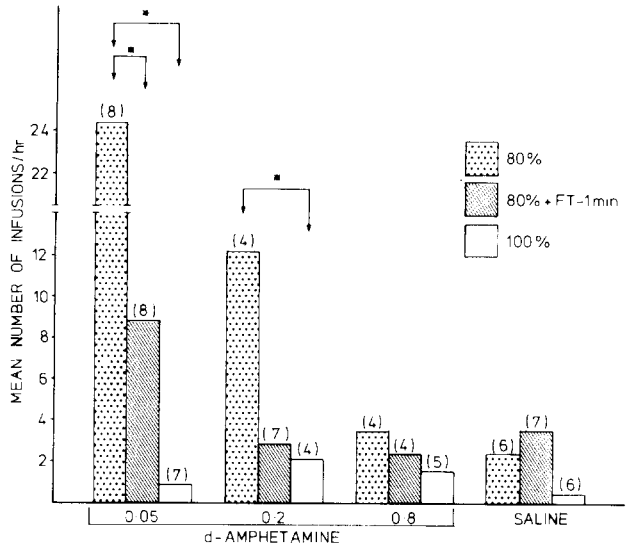

FIG. 1. The overall means of infusion for the saline and d-amphetamine groups under the three schedule conditions. *Significant difference, $p<0.05$, Scheffé *post hoc* test. ( ) Number of animals.

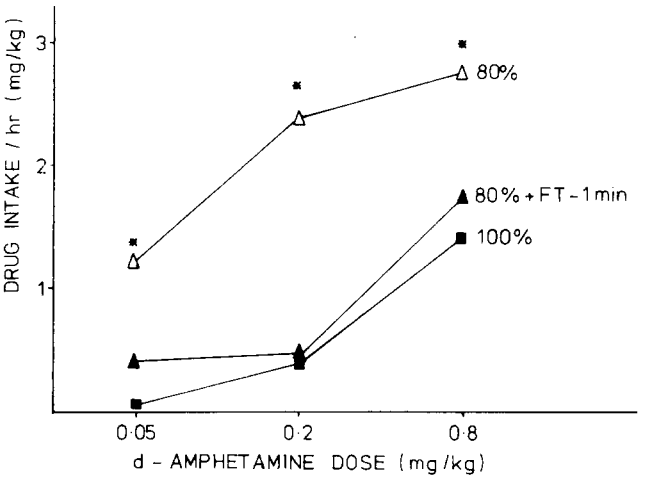

FIG. 2. The mean number of calculated drug intake of d-amphetamine 0.05, 0.2 and 0.8 mg/kg across the three schedule conditions. *Significant difference, $p<0.05$, Scheffé *post hoc* test.

0.05 mg/kg, animals at 80% body weight condition without a schedule self-induced significantly more d-amphetamine than animals at 80% body weight with an FT-1 schedule and 100% body weight condition. At dose of 0.2 mg/kg, 80% body weight animals without a schedule self-injected a significantly higher amount of amphetamine than 100% body weight animals, $p<0.05$. There were no other significant effects among the three conditions studied when the other doses of amphetamine or saline were compared (see Fig. 1).

The findings thus indicate that food deprivation and low doses (e.g., 0.05 mg/kg) of amphetamine interact to produce high rates of amphetamine self-injection. It is also important to note that there is a trend for an inverse relationship between amphetamine dose level and the rate of self-injection. Amphetamine 0.05 mg/kg seems to be the optimum dose in this study.

Figure 2 illustrates the calculated amount of amphetamine intake by animals under the three schedule conditions.

A two-way ANOVA revealed significant main effects of drug treatments, $F(2,45)=8.711$, $p<0.01$ and schedule conditions, $F(2,45)=9.280$, $p<0.01$. However, the interaction between drug treatments and schedule conditions was not significant, $F(4,45)=1.314$, $p>0.05$. These results suggest that the calculated level of drug intake is significantly different for the three schedule conditions when different doses of amphetamine were used. Post hoc Scheffé analysis showed that only animals at the 80% reduced body weight condition without a schedule at all dose levels examined self-administered significantly more amphetamine than the 80% body weight with a schedule and 100% body weight animals, $p<0.05$.

In summary, these data show that the rate of amphetamine intake is significantly greater in 80% body weight animals and also, this intake is not kept constant through the three doses tested.

DISCUSSION

The results from the present study show that animals self-inject d-amphetamine, at 80% reduced body weight condition without a schedule, at a significantly greater rate than animals at 80% body weight on an FT-1 and on 100% body weight condition without a schedule at all three doses used in this experiment. The highest rate of self injection occurred when the 0.05 mg/kg infusion of amphetamine was available (Fig. 1). The present findings also suggest that the 80% body weight animals showed an inverse relationship between dose of amphetamine and response-produced infusions per hour, thus confirming other studies using the same drug [8, 9, 17]. However, when these data were analysed as a calculated drug intake per hour (see Fig. 2), the total amount of drug intake was not constant across the three dose levels and the three conditions examined.

Analysis of the water intake data in the home cage showed that amphetamine did not produce significant differences between groups. The animals were kept at constant body weight under all deprivation conditions and also appeared healthy throughout the course of the study. Stereotyped behaviour was observed in most of the animals after drug injections. The activity consisted mainly of sniffing and licking the floor.

EXPERIMENT 2
SELF-INJECTION OF D-AMPHETAMINE 0.05 MG/KG AT 90% REDUCED BODY WEIGHT WITH AN FT-1 SCHEDULE AND WITHOUT A SCHEDULE

In the previous experiments it has been shown that animals at 80% body weight self-injected significantly large amounts of amphetamine and thus suggested that body weight reduction seems to play an important role in the self-injection of d-amphetamine. In order to further explore the body weight factor, animals in this experiment were at 90% of their normal body weight, with or without an FT-1 food delivery schedule and amphetamine 0.05 mg/kg was made available for self-administration.

Animals

Fifteen Wistar male rats were used in the present study. They were reduced to 90% body weight prior to surgery and then maintained at that weight throughout the experiment.

Procedure

The apparatus and the procedure were similar to that used in Experiment 1.

Rates of self-injection of amphetamine 0.5 mg/kg under 80% body weight plus FT-1, 80% and 100% body weight conditions were again compared to the rates of animals at 90% reduced body weight with and without schedule conditions.

The solution of amphetamine 0.5 mg/kg/infusion was prepared prior to each test session as previously described by dissolving it in 0.9% sterile saline.

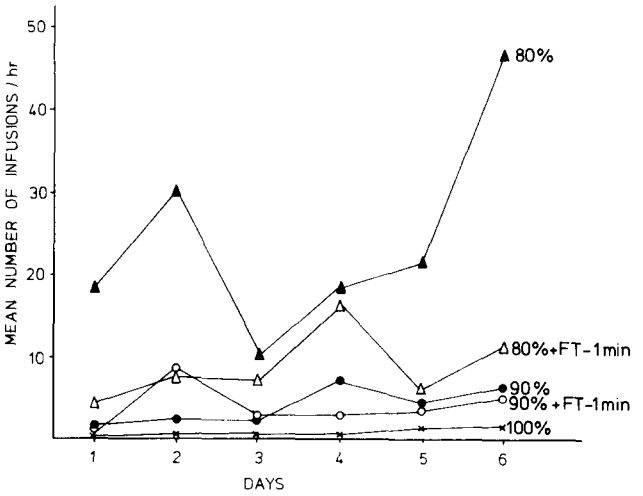

FIG. 3. The mean number of infusions for d-amphetamine 0.05 mg/kg for each session of the five groups of animals.

RESULTS

The data from this experiment were combined with the results from Experiment 1 and are shown in Fig. 3. The results of a two-way ANOVA showed significant main effects for schedule conditions, $F(4,33)=5.303$, $p<0.01$, and days, $F(5,20)=3.027$, $p<0.01$. The interaction between schedule conditions and days was not significant, $F(20,165)=1.525$, $p>0.05$. These findings suggest that the rate of self-injection of d-amphetamine for the five schedule conditions were significantly different from each other. Table 1 summarizes the results of the overall mean number of infusions/hour for the five scheduled conditions. Independent Student t tests showed that animals under 80% reduced body weight and without an FT-1 schedule condition self-injected significantly more amphetamine than animals under the other four conditions (see Table 1). There was no significant difference for the rate of self-injection between the 90% reduced body weight animals with and without FT-1 schedule conditions.

However, when the self-injection rate of the animals under 80% body weight with an FT-1 condition was compared to the self-injection rate of the animals under 90% body weight with an FT-1 condition, the difference was not statistically significant, suggesting that addition of an FT-1 schedule under these body weight reduced conditions is not an important factor in inducing large rates of self-injection of amphetamine.

TABLE 1

OVERALL MEAN NUMBER OF INFUSIONS PER HR FOR d-AMPHETAMINE 0.05 MG/KG AND SALINE

	Drug and Dose (mg/kg)	Body Weight and Schedule	Overall Mean Number of Infusions per Hr ± SE
(6)	Saline	100%	0.44 ± 0.19
(7)	d-Amphetamine 0.05	100%	0.83 ± 0.22
(8)	d-Amphetamine 0.05	90%	4.06 ± 2.25
(7)	d-Amphetamine 0.05	90% + FT-1	4.80 ± 2.10
(8)	d-Amphetamine 0.05	80%	24.36 ± 7.41
(8)	d-Amphetamine 0.05	80% + FT-1	8.80 ± 2.97

( ) Number of animals.
* Difference between groups is statistically significant ($p<0.05$, at least).
† Difference between groups is not statistically significant.

DISCUSSION

The findings of Experiment 2 indicate that for self-administration of amphetamine the food deprivation level is an important factor and that the addition of an FT-1 schedule did not increase significantly the rate of self-injection. This finding is in contrast to the results of previous studies [5,6] with nicotine, methadone and heroin using this method. In these studies [5,6] FT-1 schedule was shown to increase significantly the rate of self-administration. It is clear from Fig. 3 and Table 1 that 80% reduced body weight animals showed a higher response rate through 6 days than animals of the 90% or the 100% body weight condition.

GENERAL DISCUSSION

The findings of the present study indicate that when animals were allowed to self-inject d-amphetamine, the highest response rates were obtained at 80% reduced body weight condition without a schedule, thus suggesting that a nutritional factor is an important variable for inducing higher intake of amphetamine. Furthermore animals under 80% body weight condition showed a well known trend for an inverse relationship between dose level of amphetamine and response rate (see Fig. 1). However, in our study the amount of drug intake did not remain constant across different doses (see Fig. 2). It is likely that the short duration of our experimental session could explain this result, since in other studies [17], the stabilized rates only occurred after the first 2 hours.

Interpretation of the present findings are difficult since d-amphetamine is considered as a positive reinforcer which induces self-administration [2, 7, 16] and also reduces food intake [1,12]. The fact that the rate of self-injection of amphetamine was increased when their body weight was lowered by food deprivation suggests that the anorexigenic effect of amphetamine is primarily responsible for the large intake. However, if so, one would expect a higher or at least a similar pattern of self-injection in animals under the same deprivation condition plus FT-1 food delivery schedule. Surprisingly, as can be seen in Fig. 2, animals at 80% body weight under FT-1 schedule showed a similar pattern to normal body weight animals at all three doses examined. It is unlikely that this lower performance of 80% body weight animals under an FT-1 schedule could be attributed to the satiation after eating pellets. Marked individual differences were seen in eating behaviour. The pellet was not always consumed on its presentation. Taking this into account and also the observation that animals under this condition ignored the bar pressing activity, it seems reasonable to suggest that the interaction between environmental factor (FT-1 schedule) and physiological state give rise to a differential sensitivity of the pharmacological property of d-amphetamine. As suggested in recent papers [13,14] the physiological imbalances arising from food deprivation results in an increase in general activity. In the present study, at 80% body weight animals without a schedule, this effect seems to be critical in initiating a steady state of responding for the reinforcing property of amphetamine. It is known that schedules when combined with body weight reduction produce quantitatively more activity than deprivation alone [14]. However, as suggested previously this "hyperactive" behaviour displayed by 80% body weight animals under an FT-1 schedule seems to induce a differential sensitivity of the pharmacological action of amphetamine, thus disrupting the drug intake response.

At this stage it is intriguing also to speculate if an early evidence described by Dews [3] and others [4,11] that amphetamine which has a "baseline dependent" or "rate-dependent" effect, is affecting specifically the schedule conditions examined in this study. Further support for this explanation comes from the findings that in schedule induced polydipsia, d-amphetamine disrupted the adjunctive drinking towards the end of each session [10]. Impairment of behaviour after intraperitoneal injection of amphetamine in animals which displayed high level of adjunctive behaviour during baseline session has also been reported [15].

In conclusion the present results show that to initiate and to maintain high levels of self-injection of d-amphetamine, the interaction of food deprivation state and pharmacological properties is critical and that this interaction pattern is different from that observed for the self-administration of nicotine, methadone and heroin where a three-way interaction involving food delivery schedules maximized drug self-injection rates [5,6].

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