REVIEW

Pharmacological Studies on Drug Dependence in Rodents: Dependence on Opioids and CNS Depressants†

Tsutomu SUZUKI

Department of Applied Pharmacology, School of Pharmacy, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142, Japan

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Abstract—Physical and psychic dependence on opioids and CNS depressants in rodents were examined using the drug-admixed food (DAF) method. A comparison of several methods for developing physical dependence on opioids was made. The DAF method has the advantage of rapidly inducing a high degree of physical dependence without causing morbidity or mortality. When morphine-dependent rats were pretreated with several opioids, naloxone-precipitated weight loss was suppressed in a dose-dependent manner. A procedure for the development of severe physical dependence on CNS depressants was also established. Drug concentrations were rapidly increased until animals showed moderate to severe CNS depression, and then this condition was maintained for at least 10 days. With this procedure, animals became severely dependent on CNS depressants. Another technique, intermittent infusion, was developed that has been used to quantify short-acting CNS depressant dependence potential. The sedative effects of pentobarbital were used as an index in the determination of the injection intervals. These results suggest that the DAF method and the new approaches are useful tools for assessing the physical dependence potential of new drugs. Moreover, oral self-administration and weight pulling procedures were utilized along with the DAF method. Procedures for the oral self-administration of opioids and CNS depressants were established. Opioid-dependent rats pulled the weight to obtain the DAF even though they had free access to normal food. This weight-pulling procedure may be useful for assessing the degree of reinforcing effects for drugs in rats.

I. Physical dependence

1. Opioid

On the basis of many years of study with the opioids, well defined methods have been developed for the assessment of physical dependence. Physical dependence on opioids can quickly be induced in rodents by a variety of techniques, including implantation of morphine pellets (1–3), infusion of opioids (4–6), treatment with slow release emulsion (SRE) of opioids (7, 8) and implantation of a reservoir of morphine solution (9). The drug-admixed food (DAF) method developed by Yanaura et al. (10, 11) is an easy way to produce physical dependence on opioids in rodents without the stress of surgery or injection. It is well-known that the degree and severity of physical dependence are influenced by dose, frequency (dosing interval) and duration of drug administration and that the frequency is the most important factor (12). From these points of view, we examined physical dependence on opioids using the DAF method.

a) Effects of dose: Rats were chronically administered 5 doses of morphine (0.0625, 0.125, 0.25, 0.5 and 1.0 mg/g of food) for 7 days by the DAF method. The assessment of

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physical dependence on morphine was carried out using withdrawal signs precipitated by naloxone (3.0 mg/kg, s.c.) challenge. The average daily morphine intake for 7 days was 5.29, 10.58, 21.49, 38.19 and 76.94 mg/kg/day in animals treated with morphine at doses of 0.0625, 0.125, 0.25, 0.5 and 1.0 mg/g of food, respectively. After the naloxone challenge, rats treated with morphine clearly showed dose-dependent withdrawal signs including diarrhea, irritability, vocalization and aggression. Furthermore, drastic losses of body weight were observed within 30 min after naloxone administration. Animals treated with the lowest dose of morphine showed a significant loss of body weight 120 min after naloxone injection, although no marked changes in body weight compared with the control were observed within 60 min. The extent of these significant losses of body weight was also correlated with the morphine doses (13).

b) Effect of duration of treatment: Suzuki et al. (14) studied the effect of duration of morphine treatment using the DAF method. Morphine groups were treated with morphine-admixed food (0.5 mg/g of food) for 1 to 7 days. After the treatment, the rats were abruptly withdrawn from morphine for 24 hr or were given naloxone (3 mg/kg, s.c.). During the treatment with morphine, the animals did not show any signs of intoxication. Morphine daily intake ranged from 39.5 to 56.4 mg/kg/day. The withdrawal of morphine from morphine-treated rats caused a marked loss of body weight, and the magnitude of body weight loss 24 hr after the withdrawal was correlated with the duration of morphine treatment. Naloxone precipitated withdrawal signs that included body weight loss, diarrhea, ptosis, teeth chattering, body shakes, salivation, nose bleed, irritability, aggression, lacrimation, marked scratching and writhing. A loss of body weight was observed in all groups and was correlated with the duration of the morphine treatment period. The incidence of the naloxone precipitated withdrawal signs was also correlated with the duration of the morphine treatment period. The withdrawal signs produced by opioid antagonists, in contrast to abrupt withdrawal, were a rapid, explosive event that condenses in a short time period the withdrawal signs of abrupt withdrawal. We also obtained similar results using codeine (15).

c) Effect of frequency of treatment: The frequency of drug treatment is the most important factor in determining the dependence potential of a drug. From this point of view, studies have been made of methods in which experimental animals can acquire a dependence on drugs under conditions of exposure to the drugs such as implantation of morphine pellet, infusion of opioids, treatment with SRE of opioids, etc. The DAF method, unlike the injection method (16), does not force the animals to ingest a given amount of drug, rather they can eat the DAF ad libitum. Continuous recording of the time course of food intake and eating behavior makes a study of such a change possible. However, as there had been no apparatus capable of recording the parameters, we developed automatic equipment which can monitor continuous variations in food intake, eating behavior and approach to food during the morphine treatment. The naive rats had a normal eating pattern, and most of the eating (87.9%) was done intermittently during the dark period. On the other hand, the rats on the morphine-admixed food (1 mg/g of food) ate the food frequently at different times throughout the day, even from the first day on the diet. The food intake in the dark period accounted for 60.3% of the total food intake per day, which was significantly lower than the food intake of the naive rats. Although the morphine-dependent rats exhibited the major part of their eating behavior during darkness (79.0% of the total food intake per 24 hr), the eating time of the morphine-dependent rats was longer than that of naive rats. These results show that the DAF method in which the rats frequently eat the morphine-admixed food rapidly produces morphine-dependent rats (17).

d) Comparisons of several methods of inducing physical dependence: Rats were treated with morphine-admixed food (0.5 and 1 mg/g of food), morphine SRE (75, 100 and 150 mg/kg) and morphine (75 mg) pellets. In the SRE and pellet methods, the typical signs of morphine toxicity, such as catatonia, exophthalmos and shallow respiratory movement, were observed 15–20 min after the

treatment, and these signs were maintained for 14–18 hr. In rats treated with SRE and pellets, plasma morphine levels reached a maximum 1 day after the treatment, and subsequently decreased, while plasma morphine levels in rats treated with DAF increased treatment period-dependently. Withdrawal signs precipitated by naloxone (3 mg/kg, s.c.) in rats treated with DAF, SRE and pellets were characterized by loss of body weight, body shakes, vocalization, diarrhea, ptosis, teeth-chattering, nose bleed, salivation and lacrimation. The intensities of naloxone-precipitated withdrawal signs reached a maximum 1–2 days after the treatment with SRE and pellets, and they were correlated with the duration of DAF treatment. Rats treated with DAF, SRE (150 mg/kg) and pellets for 3 days manifested loss of body weight, diarrhea, etc. after the naloxone challenge (Fig. 1 and Table 1). Thus, physical dependence on morphine can be induced rapidly by these three methods. However, the SRE and pellet methods induced morphine toxicity, and it was difficult to maintain physical dependence on morphine in rats. These results suggest that the DAF method is the best method for inducing physical dependence on morphine in rats (18).

e) New approaches for assessment of physical dependence on opioids: Three methods for assessment of physical dependence on opioids in rats have been developed. First, the relationship between physical dependence on opioids and the osmotic fragility of the erythrocyte membrane in rats was examined. The osmotic fragility of erythrocyte membranes in morphine-dependent rats was significantly enhanced, compared with that of naive rats. By withdrawing morphine or treating the rats with opioid antagonist, the osmotic fragility was significantly enhanced more than in the morphine dependent state. When the morphine-withdrawal rats were

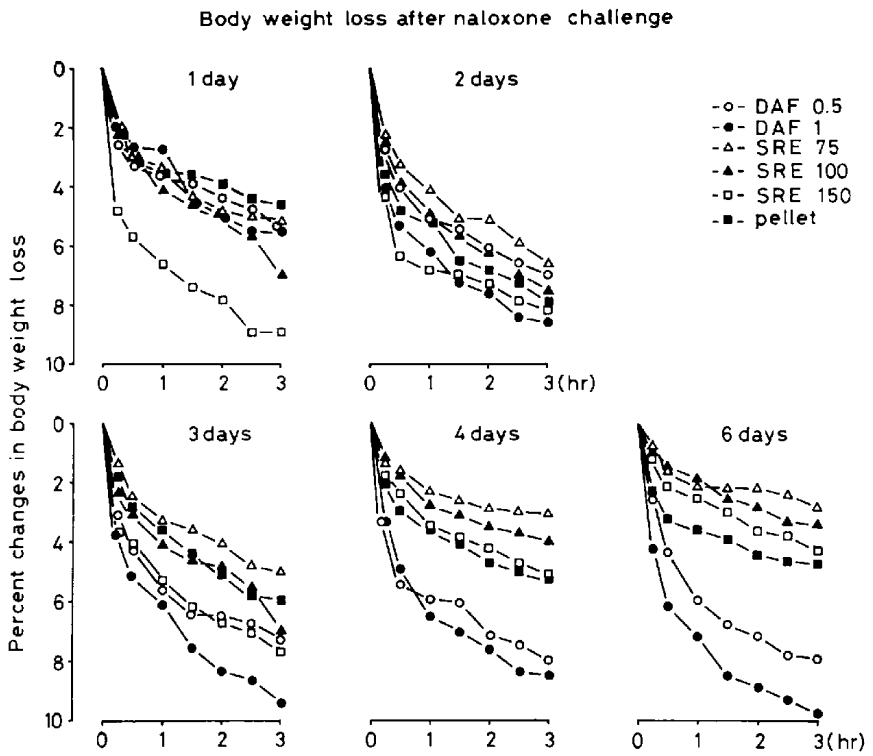

Fig. 1. Percent changes in body weight after naloxone (3 mg/kg, s.c.) challenge in rats treated with morphine-admixed food, morphine slow release emulsion and morphine pellets. Each plot represents the mean of 5–6 animals.

Table 1. Withdrawal signs induced by naloxone (3 mg/kg, s.c.) in rats treated with morphine by drug-admixed food (DAF), slow release emulsion (SRE) and pellet methods

Duration of treatment (days)	Withdrawal signs	DAF		SRE			Pellet
		0.5 (mg/g food)	1	75	100 (mg/kg)	150	75 (mg)
1	Diarrhea	+++	++	++	++	+++	+
	Ptosis	+++	++	+++	+++	+++	+++
	Body shakes	++	+++	+++	+++	+++	++
	Teeth-chattering	++	++	+++	+++	+++	+
	Vocalization	–	–	+++	++	+++	++
	Salivation	++	++	+++	++	++	++
	Nose bleed	–	+	+	++	+	–
	Lacrimation	–	–	+	++	++	++
2	Diarrhea	+++	+++	++	+++	+++	++
	Ptosis	+++	+++	++	+++	+++	+++
	Body shakes	+++	+++	+++	+++	+++	+++
	Teeth-chattering	++	+++	+++	+++	+++	++
	Vocalization	+	+++	++	+++	+++	+++
	Salivation	++	++	+	+	+++	+++
	Nose bleed	+	++	+	++	–	++
	Lacrimation	–	+	+	+	+	+
3	Diarrhea	+++	+++	++	++	++	++
	Ptosis	+++	+++	++	+++	++	+++
	Body shakes	+++	+++	++	+++	++	+++
	Teeth-chattering	++	+++	++	++	++	++
	Vocalization	+++	+++	++	–	++	+++
	Salivation	++	++	–	+	–	++
	Nose bleed	–	++	+	–	+	–
	Lacrimation	–	++	+	–	–	+
4	Diarrhea	+++	+++	++	++	+++	+++
	Ptosis	+++	+++	+++	+++	++	+++
	Body shakes	+++	+++	++	++	++	+++
	Teeth-chattering	+++	++	–	–	+	+
	Vocalization	+++	+++	++	++	++	+++
	Salivation	++	++	–	+	++	++
	Nose bleed	+	+	–	–	+	+
	Lacrimation	–	++	–	+	+	++
6	Diarrhea	+++	+++	+	–	+++	++
	Ptosis	+++	+++	++	+++	++	+++
	Body shakes	+++	+++	+	+	+	++
	Teeth-chattering	++	++	–	–	+	+
	Vocalization	+++	+++	++	++	+	+++
	Salivation	++	++	–	–	–	+
	Nose bleed	–	+	–	–	–	–
	Lacrimation	+	++	–	–	–	+

+++ Sign present in 100% of animals, ++ sign present in 50–99% of animals, + sign present in 1–49% of animals, – sign absent.

again given the morphine-admixed food, the osmotic fragility recovered to morphine-dependent levels (19). Secondly, we found that chronic administration of morphine caused a marked decrease in the urinary excretion of low molecular weight protein (LMWP) which exists only in male rats. The amount of excreted LMWP was observed to

decrease 3 days after giving morphine-admixed food, and to recover to control levels within 6 days after morphine withdrawal. Morphine produced a dose-related decrease in LMWP excretion, which was correlated with the intensity of withdrawal signs after naloxone challenge (13, 20). Thirdly, rats treated with morphine exhibited weight loss upon injection with naloxone. When morphine-dependent rats were injected s.c. with morphine, codeine, meperidine and pentazocine 30 min before the naloxone injection, these drugs significantly suppressed the naloxone-precipitated loss of body weight in a dose-dependent manner (21). These results suggest that the three approaches are suitable for assessing physical dependence on opioids.

2. CNS depressants

Studies of physical dependence on CNS depressants have chiefly been conducted on monkeys (22), dogs (24) and cats (24). Because of their resemblance to those in man, barbiturate withdrawal signs in dogs and monkeys have been useful for preclinical tests of drugs, while rodents have not proved to be as useful, although they have the advantage of lower cost. A barbital withdrawal convulsion can be induced in rats as first demonstrated by Crossland and Leonard (25). We have developed the DAF method for assessing physical dependence on CNS depressants in rats (11, 26). As a result, rats, like dogs and monkeys, are suitable experimental animals for tests in early stages of dependence liability, and the administration of DAF is a useful method of developing physical dependence on barbiturates. However, the incidence of severe physical dependence manifested by withdrawal convulsions has not been shown with short-acting barbiturates, e.g., pentobarbital, and benzodiazepines, in rodents. A procedure for the development of severe physical dependence on CNS depressants was examined.

a) Effects of dose and duration of treatment: The production of physical dependence on CNS depressants is also directly related to the dose, dosing interval, duration of treatment and route of administration. Boisse and Okamoto (24) established the "chronically equivalent" dosing procedure, in which the dose is continually adjusted in order to achieve a relatively stable degree of drug effect throughout the drug-administration period. Utilizing this technique, dependence, as evidenced by withdrawal signs upon withdrawal, occurs with chronic barbital or pentobarbital administration in cats; the dependence is graded in intensity, and its magnitude is a function of both the dose and duration of chronic drug treatment. On the other hand, severe physical dependence on methaqualone, as manifested by spontaneous convulsions after its withdrawal, has not been documented, suggesting that physical dependence on methaqualone, a non-barbiturate sedative-hypnotic agent, is less frequent and of a milder type. Moreover, Yanagita and Miyasato (27) found that methaqualone does not produce any physical dependence in monkeys. We developed an escalating dose schedule for assessing physical dependence on short-acting barbiturates, non-barbiturates and benzodiazepines in rats. Drug concentrations in DAF were rapidly increased until animals showed moderate to severe CNS depression, with motor incoordination as an index of the development of tolerance, and then this condition was maintained for at least 10 days (Fig. 2). With this procedure, the animals became severely dependent on pentobarbital (28, 29), methaqualone (40, 41) and diazepam (42), and withdrawal convulsions can be induced. These results suggest that an escalating dose schedule is one more important factor for developing physical dependence on CNS depressants in rats.

b) New approach for assessment of physical dependence on CNS depressant: The development of physical dependence on CNS depressants appears to be a function of continued depression of the CNS which is achieved by a sufficient and constant level of drug applied for a sufficiently long duration. We have developed a new intermittent intravenous infusion method for the chronic administration of pentobarbital. Rats were injected with pentobarbital (20 mg/kg/injection) through an implanted intravenous cannula. The rats were allowed to receive an injection after the completion of a fixed amount of behavioral activity counted from the preceding injection, and therefore, the sedative effects of pentobarbital were used as

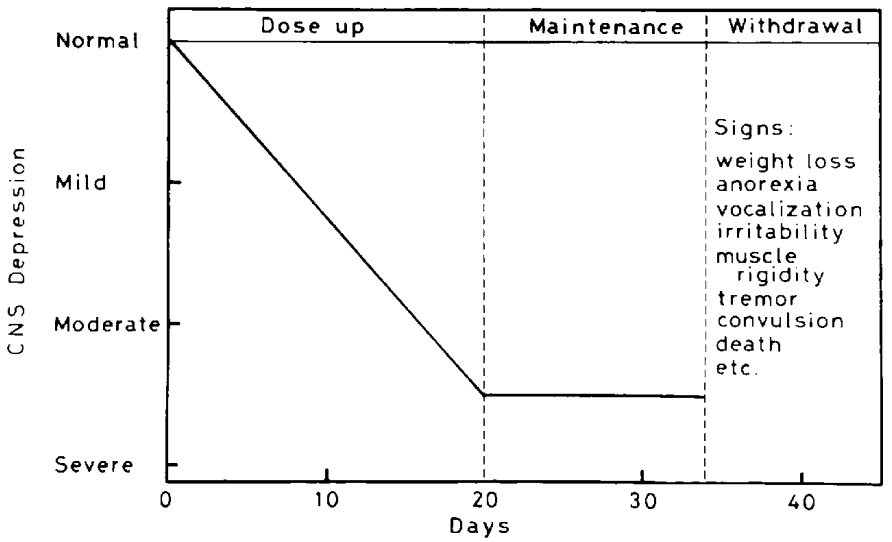

Fig. 2. Dosage schedule for dependence on CNS depressants, characteristics of DAF (Drug-Admixed Food) method for exposure of the drug to animals. The magnitude of CNS depression was evaluated by the rotarod performance test.

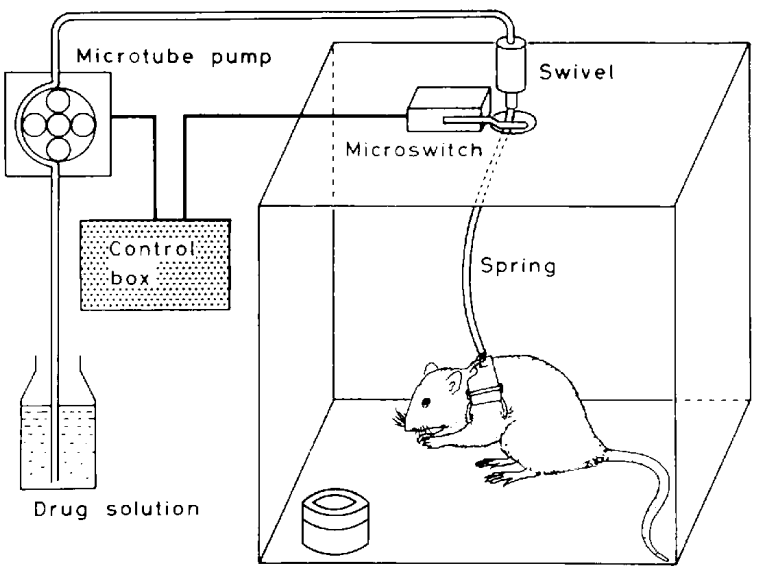

Fig. 3. Apparatus for drug injection.

an index for determining the injection intervals (Fig. 3). Two potential problems associated with chronic administration of pentobarbital are the rapid induction of microsomal drug-metabolizing enzyme systems and the rhythm in response to pentobarbital. Since the drug effect was used as an index to determine when to infuse the drug, these problems were solved. During pentobarbital treatment, the number of pentobarbital injections per day rapidly increased and stabilized on the third to fifth day. Upon withdrawal, the rats who were maintained on pentobarbital administration of more than 40 injections/day for at

least 10 days manifested withdrawal signs which included spontaneous convulsion. This method has proven to be a useful tool for the study of CNS depressants dependence in rats (33).

II. Psychic dependence

Laboratory methods of assessing the reinforcing effect of drugs in animals have been developed and are now being used for the prediction of the dependence potential in man. Oral (34–37), intragastric (38), intravenous (39–41), intraperitoneal (42) and intraventricular (43) self-administration routes have been used. In oral self-administration, among the most convincing techniques are those in which animals are given opportunities for administering drugs to themselves, either by eating the drug mixed with their diet, by drinking drug solutions, or by pressing levers which deliver DAF pellets or drug solutions. This section reports new findings made while we were developing a method for oral drug self-administration by rats.

1. Preference for drugs

The two bottle or two cup method is used to determine the preference for drugs. The two bottle method provides a situation where there are two kinds of liquids between which the rat can choose one and thereby show a preference. This situation is similar to the case of the two cup method. We utilized both the two and five cup methods and observed the preference for morphine in rats noncontingently pretreated with the drug and the increase in preference for the drug when the feeding was limited. In morphine pretreated rats, the preference for morphine was 61.2± 3.0% with the five cup method and 61.8± 3.3% with the two cup method during the choice trials. We also treated rats with morphine using the CFF schedule. The schedule consisted of one choice trial between the intake of normal food and DAF followed by two consecutive forced trials, in which the rats were forced to take the DAF only. The preference rate for morphine during choice trials rapidly increased in the five cup method and stabilized at approximately 60%. Findings with the two cup method were similar. After the preference for morphine was stabilized at 60%, morphine was given s.c., and it was found that the preference rate was dependent on the dose injected. When the concentration of DAF was changed, the preference rate changed in parallel with the concentration (37, 44). Findings with codeine were similar (45). These studies clearly demonstrate drug-seeking behavior. Therefore it is concluded that opioid treatment enhances spontaneous intake of opioid-admixed food.

The preference for drugs was studied by means of the antagonistic conflict between the positive drive of drug intake and the negative drive of weight pulling in rats. An apparatus was developed in which rats were compelled to pull the weight for the intake of DAF. The experiments began with the pre-administration of the drug through the repetition of the CFF schedule. In the test trial, the findings were that those rats which had already shown drug seeking behavior toward morphine, codeine or cocaine pulled a weight to obtain each drug and that the reinforcing effects of opioids on the drug seeking behavior depended on the treatment period of these drugs, and the reinforcing effects of cocaine depended on the cocaine concentration. The reinforcing effect of codeine was weaker than that of morphine. It is concluded that the weight pulling method is sufficient to evaluate qualitatively and quantitatively the reinforcing effects of opioids and cocaine in rats, and this method may be useful for the prediction of dependence potential in man (45–47).

2. Oral self-administration

Oral self-administration techniques have been developed by Meisch and his colleagues (36, 48, 49) that permit the establishment of orally delivered drugs (drug solution) as reinforcers for both rats and monkeys. This procedure parallels intravenous drug self-administration research. We have developed another oral self-administration using the DAF method. Pellet food (45 mg) that was produced by mixing drug into normal food was prepared. A steel wall of the experimental chamber was equipped with two levers, food trays and small lights. Normal pellet was supplied from one food tray and drug pellet from the other tray. The experiments began with the pre-administration of drug through the repetition of the CFF schedule. Sessions were

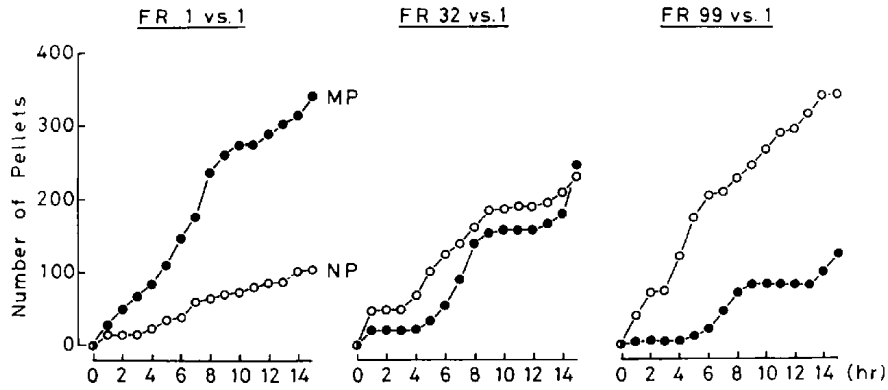

Fig. 4. Mean food pellet deliveries cumulated at 1 hr intervals over 15 hr sessions are presented. The fixed ratio (FR) schedules for morphine pellet (MP) and normal pellet (NP) are indicated by underlining, i.e., 1, 32 and 99 for MP and 1 for NP. Each plot represents the mean of 6 rats.

15 hr in duration. After the pretreatment, the size of the fixed ratio (FR) schedule for the drug was increased gradually, FRs 1, 2, 4, 8, 16, 32, 64 and then 99, while that for the normal pellet remained at FR 1. Increases in the FR values produced increases in response rates and decreases in the number of drug pellets (Fig. 4). In this procedure and the techniques of Meisch and Carroll (36) with modification for the DAF, morphine, codeine and pentobarbital were established as a reinforcer for rats (47, 50).

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