PREFERENCE FOR MORPHINE AND DRUG-SEEKING BEHAVIOR IN MORPHINE DEPENDENT RATS

Saizo YANAURA and Tsutomu SUZUKI
Department of Pharmacology, Hoshi College of Pharmacy,
Shinagawa-ku, Tokyo 142, Japan

Accepted March 15, 1978

Abstract—We have already reported that morphine pretreated rats prefered morphine-admixed food during choice trials with the two cup method. In the present work, we utilized both the five and two cup methods and observed the preference for morphine in rats forcedly pretreated with the drug and the increasing rate of preference for the drug in rats where the feeding time was limited. In morphine pretreated rats, preference rate 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. In rats that were limitedly treated with morphine, each preference rate for morphine during choice trials rapidly increased in the five cup method, i.e. 5.5→14.4→31.7→43.6→61.2%, and the preference rate for morphine stabilized at approximately 60 percent. Findings with the two cup method were similar. After the preference for morphine was stabilized at the 60 percent level, morphine was given subcutaneously and it was found that the preference rate was dependent on the dose injected. When the drug-admixed concentration was changed from 1 mg/g food to 0.5 and 2 mg/g food, the preference rate changed in parallel with the concentration. When the number of food cups containing morphine-admixed food was changed from 1/5 to 2/5, 3/5 and 4/5 food cup, the preference rate was not effected. These studies clearly demonstrate drug-seeking behavior in rats. In the process of preference for morphine, morphine treatment enhances spontaneous intake of morphine-admixed food.

Self-administration of drugs is extensively used in screening methods for psychic dependence liability (1). Peroral (2–7), intragastric (8–10), intravenous (1, 11), intraperitoneal (12) and intraventricular (13) self-administration routes have been used. Nichols et al. (2), Nichols and Davis (3), Wikler et al. (4) and Kumar et al. (5) reported that rats will drink a morphine solution despite its aversive taste. We have demonstrated that rats treated with opiates, barbiturates and minor tranquilizers admixed food (DAF method) became physically dependent on each drug for 7 days (14–16) and also showed preference for each drug during a choice trial (6, 7). In the studies of peroral self-administration, 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 liquid between which the rat can choose the preferred one. This situation is similar to the case of the two cup method. Kagawa (17) reported that alcohol preference depended on the number of bottles and so the two bottle method was not suitable for the test of alcohol preference.

In the present study, we utilized both the two cup and five cup methods by which five food cups are set in a cage instead of two cups. We, then observed the preference for morphine in rats forcedly pretreated with the drug and the increasing rate of preference for the drug...
in rats where the feeding time was limited. In the five cup method, the preference for morphine was also determined when morphine dependent rats were injected with morphine and the number of cups containing morphine-admixed food and concentration of morphine-admixed food were changed. The relation of the preference to morphine dependence is also discussed.

MATERIALS AND METHODS

Each group included 6 male Sprague-Dawley strain rats (Charles River Japan, Inc., Kanagawa), 5 weeks of age which were housed individually in cages (36 × 24 × 17 cm or 21 × 25 × 15 cm) with five or two food cups equi-distant from the water bottle. The position of the food cup containing the drug was changed daily. Morphine hydrochloride and quinine sulfate were used in this experiment. Each drug was admixed with rat food (CA-1 powder, CLEA Japan, Inc., Tokyo) at drug/food ratios of 0.5 mg/g, 1 mg/g and 2 mg/g.

EXPERIMENT 1. (free feeding group)

All animals ingested morphine at the rate of 1 mg/g of food for 3 weeks. The pretreatment period was followed by 3 days withdrawal period during which only ordinary food was provided. This withdrawal served to motivate desire for the drug. The drug-admixed food was again given for 1 week. These procedures were common in the five and the two cup methods, namely only one type of food was used for both methods. The behavior of these pretreated rats was observed during the daily choice trial for 1 week (one cup containing drug-admixed food vs. one cup with the two cup method or four cup with the five cup method containing ordinary food). During these trials, the preference rate for the drug was compared between the morphine and quinine groups as well as the difference between the five and two cup methods. Preference rate was calculated as follows:

\[
\text{Preference rate (\%)} = \frac{\text{drug-admixed food intake (g)}}{\text{total food intake (g)}} \times 100
\]

In this experiment, rats had free access to food and water. Body weight and food intake were measured daily at 16:00. In the control experiments, quinine was used instead of morphine.

EXPERIMENT 2. (limited feeding group)

Rats were allowed access to food only between 9:00-17:00, but access to water was free. Body weight was measured daily at 9:00 and 17:00. Total food intake during 9:00-17:00 was also recorded daily. Actual daily drug intake was calculated in mg/kg of body weight at 17:00/day.

1) Changes in preference rate for drug during choice trials.

The experiment was performed as follows: one experimental session consisted of one choice trial (the rats can choose the preferred one between the two or among the five) and then two forced trials (in this case the only one cup containing drug-admixed food was set). After 7 sessions the choice trial was performed daily for 1 week. Increasing trend of preference rate for morphine or quinine was compared between the five cup and two
cup methods.
2) Changes in preference rate for morphine in the five cup method after morphine injection.

In the five cup method, the preference for morphine was stabilized at approx. 60% with all rats. Twenty-four rats were separated into four groups and were started on the choice trial 30 min after being given morphine (10, 30 and 50 mg/kg) or saline s.c. In the choice trial, preference rate for morphine, total food intake and body weight were measured and compared among the four groups.

3) Relation of preference rate for morphine to the concentration of morphine admixed in the five cup method.

Six rats with a preference rate for morphine which was stabilized at approx. 60% in the 14 day choice trial were used for the five cup method experiment. During these trials, three different concentrations of morphine-admixed diets were provided, i.e. 1 mg/g food, 0.5 mg/g food and 2 mg/g food. The choice trials in each concentration were given for 6 days, respectively. During these trials, preference rate for morphine, total food intake, morphine intake and body weight were measured and compared with each concentration.

4) Influence of the number of food cups containing morphine-admixed food on preference rate for morphine in the five cup method.

In the five cup method, six rats with a preference rate for morphine which was stabilized at approx. 60% in the choice trials were used. The number of cups containing morphine-admixed food was changed to 2, 3 and 4 cups among 5 cups. In the choice trials, preference rate for morphine, total food intake, morphine intake and body weight were measured and compared in each treatment.

RESULTS

EXPERIMENT 1. (Free feeding group)

When five cups contained non-drug admixed food, six naive rats ate the same amount from each cup, within a 10–30% range. As for the probability with the two cup method, it would be expected that rats would choose morphine- or quinine-admixed food between the two at 50%. However, naive rats rarely ate morphine- or quinine-admixed food as

| Method        | Naive rats | Drug treated rats |
|---------------|------------|-------------------|
|               | Quinine    | Morphine          | Quinine    | Morphine          |
| Five cup method | 2.0±0.7   | 0.9±0.3           | 3.3±0.7   | 61.2±3.0a,b      | (%)       |
|               | 2.4±0.9   | 0.6±0.3           | 2.3±0.6   | 50.9±4.8a,b      | (mg/kg/day) |
| Two cup method | 2.9±1.2   | 2.0±0.5           | 3.6±1.1   | 61.8±3.3a,b      | (%)       |
|               | 4.8±2.1   | 2.4±0.6           | 2.7±0.8   | 52.5±2.8a,b      | (mg/kg/day) |

a: significantly different from the quinine group (P<0.001). b: significantly different from the group of naive rats (P<0.001). Each value represents the mean±S.E.M. of 6 rats for 1 week.
observed in choice trials for 1 week in both the five and two cup methods (Table 1.). On the contrary, when rats were forced to eat morphine-admixed food for 4 weeks, then put on daily choice trials for 1 week, the preference rate for morphine markedly increased, i.e. 61.2±3.0% (mean±S.E.M.) in the five cup method and 61.8±3.3% in the two cup method (Table 1. and Fig. 1.). These values were significantly different from the values of naive rats (P<0.001). In the quinine treated rats, however, preference rate for quinine did not differ from the values of naive rats (Table 1.). During the choice trials, the preference rate for morphine or quinine was much the same with both the five and two cup methods. During the morphine treatment, food and morphine ingestion was approx. 20 g/day and 100 mg/kg/day in both methods, respectively. On the second day of morphine withdrawal after morphine treatment for 3 weeks, weight loss was approx. 11% in both methods. In the quinine group, food and quinine ingestion was similar to the morphine group. However, weight loss did not occur during the quinine withdrawal period.

In the first of the choice trials for 1 week in the morphine groups, body weight and total food intake decreased slightly. By comparison, these decreasing rates of body weight and total food intake on the last morphine treatment day were 2.0±2.4% and 29.2±12.3% in the five cup method, and 3.0±0.9% and 14.6±3.5% in the two cup method, respectively. After the second trial, however, such changes were not observed. In the last one week of morphine treatment (for 4 weeks), mean morphine intake was 76.6±5.9 mg/kg/day in the
five cup method and $78.7 \pm 2.0$ mg/kg/day in the two cup method, while mean morphine intake during the choice trials was $50.9 \pm 5.9$ mg/kg/day in the five cup method and $52.5 \pm 2.8$ mg/kg/day in the two cup method (Fig. 1.). In the quinine group, body weight and total food intake did not change with either method during the choice trials (Fig. 1.).

**EXPERIMENT 2. (limited feeding group)**

Figure 2 shows body weight and total food intake changes in the free feeding group and the limited feeding group in naive rats. Growth curve and total daily food intake inhibitions were observed in the limited feeding group.

1) Changes in preference rate for the drug during the choice trial.

Figure 3 shows preference rate for drug-admixed food and drug intake. Preference rate for morphine rapidly increased in every choice trial of each session, i.e. $5.5 \rightarrow 14.4 \rightarrow 31.7 \rightarrow 43.6 \rightarrow 61.2\%$ in the five cup method and $13.4 \rightarrow 15.8 \rightarrow 25.9 \rightarrow 41.1 \rightarrow 69.1\%$ in the two cup method, after which these preference rates stabilized at approx. 60%. During daily choice trials, preference rate for morphine also stabilized at approx. 60%. Morphine intake rapidly increased as the result of increment of preference rate for morphine, i.e. $6.6 \rightarrow 11.8 \rightarrow 22.2 \rightarrow 54.1$ mg/kg/day in the five cup method and $13.1 \rightarrow 12.7 \rightarrow 23.3 \rightarrow 54.3$ mg/kg/day in the two cup method, after which morphine intake stabilized at approx. 50 mg/kg/day in each method. Mean morphine intake during daily choice trials for 1 week was $51.6 \pm 1.6$ mg/kg/day in the five cup method and $50.6 \pm 2.7$ mg/kg/day in the two cup method. On the contrary, preference rate for quinine did not increase with either method in all choice trials. Mean quinine intake during daily choice trials for 1 week was $7.4 \pm 3.3$ mg/kg/day in the five cup method and $7.1 \pm 1.6$ mg/kg/day in the two cup method.

![Fig. 2. Growth curve and food intake of free feeding group (○) and limited feeding group (●). Zero point on the axis of abscissa corresponds to four weeks from birth. Each plot represents the mean of 6 rats.](image-url)
2) Changes in preference rate for morphine in the five cup method after morphine injection.

In rats in which the preference rate for morphine was stabilized at approx. 60%, morphine or saline was given s.c. 30 min before the choice trial. In the choice trial, preference

Fig. 3. Preference rate for drug and drug intake in each choice trial of the limited feeding group of five cup and two cup methods. Each plot represents the mean of 6 rats. DAF: drug-admixed food. —●—: morphine group, —○—: quinine group.

Fig. 4. Effects of morphine injection (s.c.) 30 min before the choice trial on preference rate for morphine in the five cup method. Left: changes in mean total food intake (open column) and morphine-admixed food intake (closed column). Each column represents the mean of 6 rats. Right: changes in mean preference rate for morphine. Each plot represents the mean of 6 rats. MAF: morphine-admixed food. Vertical bars represent standard errors.
rate for morphine was negatively related to the dose, that is 45.8 - 6.1% in the morphine 10 mg/kg group, 24.7 ± 4.3% in the morphine 30 mg/kg group, 16.7 ± 3.5% in the morphine 50 mg/kg group and 67.3 ± 8.7% in the saline group (Fig. 4.). However, body weight and total food intake changed little in each group.

3) Relation of preference rate for morphine to the concentration of morphine admixed in the five cup method.

When the concentration of morphine-admixed food in choice trials was changed, preference rate for morphine was 65.8 ± 1.8% in 1 mg/g food, 69.5 ± 3.3% in 0.5 mg/g food and 35.8 ± 2.2% in 2 mg/g food. Mean morphine intake during the choice trials for 1 week was 43.7 ± 2.3, 22.8 ± 2.0 and 41.0 ± 1.7 mg/kg/day, respectively. However, body weight and total food intake showed little change during the choice trials (Fig. 5.).
4) Influence of the number of food cups containing morphine-admixed food on preference rate for morphine in the five cup method.

When one of the five cups contained morphine-admixed food in a choice trial, preference rate for morphine was 67.1±7.1 %. The preference rate for morphine was 69.5±7.7 % in two among five cups, 74.4±6.5 % in three among five cups and 68.7±9.1 % in four among five cups (Fig. 6.). The morphine intake was 42.2±5.7, 48.2±7.1, 52.7±8.6 and 46.1±8.9 mg/kg/day, respectively. Rats ate the same amount of morphine-admixed food from each food cup containing morphine-admixed food in the choice trials. However, there was little change in body weight and total food intake.

DISCUSSION

Nichols et al. (2), Nichols and Davis (3) and the authors (6, 7) reported that morphine treated rats preferred morphine solution or morphine-admixed food despite its aversive taste. These workers utilized the two bottle or two cup method. But whether the number of vessels affects the preference for drug was not proved. In the present study, we used the five cup method in order to clearly observe drug-seeking behavior and to compare the two methods.

We found that naive rats took non-drug admixed food from each cup in both the five or two cup methods. These naive rats, however, rarely chose the morphine- or quinine-admixed food in either method. These results coincide with the reports of Nichols et al. (2) and Kumar et al. (5) regarding the two bottle method. On the contrary, morphine treated rats markedly increased their preference for morphine in the choice trials. The rats searched for the one food cup containing morphine-admixed food, then ate the contents, thus indicating a clear drug-seeking behavior. In the choice trials, the preference rate for morphine in the five cup method was almost equal to findings with the two cup method and we could conclude that the rats took morphine-admixed food willingly, and apparently could determine the self-control maintenance dose in morphine dependence. However, preference rate for quinine with either method did not appear to be influenced by quinine pretreatment. In the morphine group, the weight loss and a decrease in food intake in the first choice trial day revealed a mild withdrawal syndrome as morphine intake decreased from approx. 75 mg/kg/day to 30-45 mg/kg/day, in both methods. However, this withdrawal syndrome was not observed after the second choice trial day, because morphine intake was stable at approx. 55 mg/kg/day in both methods. This result indicates that the maintenance dose of morphine dependence at this stage is approx. 55 mg/kg/day.

Kumar et al. (5), Stolerman and Kumar (18, 20) and Stolerman et al. (19) reported that a preference for morphine could be induced by the two bottle method even without pre-medication as the rats were accustomed to satisfying their normal thirst craving during a limited time daily, by substituting morphine solution for the water normally given. We modified this method, i.e. seven hours drinking time daily to eight hours feeding time daily, a drug solution to a drug-admixed food and the two bottle to the five or two cup method. Preference for morphine was rapidly acquired in our experimental animals as compared to
the results reported by Kumar et al. (5) and preference rate for morphine was stable at approx. 60% from the fifth choice trial day. Preference rate for quinine in the two cup method was similar to the result of Kumar et al. However, preference rate for quinine in the five cup method was at a low level in comparison with the two cup method. In the first choice trial day, preference rate for morphine in the two cup method was also higher than in the five cup method. These results suggest that the five cup method provides a less casual feeding of morphine-admixed food in comparison with the two cup method. We did observe however, the rapid increase of preference rate for morphine during the first five experimental sessions in which the forced treatment of morphine was included, therefore we concluded that this treatment might enhance the preference for morphine. Fig. 7 shows body weight and total food intake in Exp. 2-1). In the second choice trial day in the morphine group, withdrawal syndrome, i.e. weight loss and decrease of food intake, were evident. The withdrawal syndrome was gradually modified with each choice trial. This result suggests that the withdrawal syndrome is one cause of the increment of preference rate for morphine. It appeared that our rats definitely had been looking for the morphine-admixed food cup among the five cups, since the fifth choice trial day.

When the preference rate for morphine was stabilized at approx. 60%, in choice trials in rats given morphine s.c., the preference rate for morphine linearly decreased in parallel with the dose, while body weight and food intake did not change with the dose (Fig. 4.). These results are consistent with those in the report of Stolerman and Kumar (18). The rats seemed to be satisfied with the morphine injection and preferred ordinary food to morphine-admixed food in the choice trial. When a concentration of morphine-admixed

![Image](image-url)
food in a choice trial was changed, rats ate the morphine-admixed food in comparable amounts to the concentration of the drug (Fig. 5.). In order to demonstrate that this response was not altered by the aversive reaction of morphine we observed that after the preference rate for morphine (1 mg/g food) was stabilized at the 60% level, morphine-admixed food was changed to morphine 0.5 mg plus quinine 0.5 mg/g food and then the preference rate increased. From our experiments we found the preference rate for morphine 1 mg/g food and that for morphine 0.5 mg plus quinine 0.5 mg/g food were equal with the naive rat which may indicate that the aversive reactions were equal for both drug-admixed foods. When morphine-admixed food was changed from 1 mg/g to 0.5 mg/g food in the choice trials, withdrawal syndrome was not observed. If such a daily choice trial is extended for 21 days, the maintenance dose of morphine required to maintain the physical dependence in rats would be approx. 25 mg/kg/day (Fig. 5.). The difference in morphine ingestion between the concentration of 1 mg/g (or 2 mg/g) and that of 0.5 mg/g was approx. 20 mg/kg/day. The withdrawal syndrome did not occur during the 0.5 mg/g dosing period. But during the 1 mg/g and 2 mg/g dosing periods the rat took more morphine per day than during the 0.5 mg/g dosing period. Such observations suggest that these excessive ingestions are not the result of physical dependence but rather a psychic one. In the five cup method, when the number of cups containing morphine-admixed food was changed from 1 to 2, 3 and 4 food cups, the preference rate for morphine changed little in the choice trials (Fig. 6.). We therefore conclude that the preference rate for morphine does not depend on the number of cups containing morphine-admixed food, while the preference for alcohol does depended on this factor (17).

The conclusions obtained in these studies are as follows: We observed drug-seeking behavior in rats using the five cup method, namely, rats preferred the cup containing morphine-admixed food. We induced in rats a preference for morphine in both experimental groups (Exps. 1 and 2). In Exp. 2, the preference for morphine increased linearly with the experimental sessions. A preference for morphine was enhanced by pre-treatment with this same narcotic. This enhancement may be the result of escape from withdrawal syndrome. Morphine dependent rats apparently can control their own required maintenance dose.

REFERENCES

1) Deneau, G.A., Yanagita, T. and Seever, M.H.: Self-administration of psychoactive substances by the monkey. A measure of psychological dependence. Psychopharmacol. 16, 30-48 (1969)
2) Nichols, J.R., Headlee, C.P. and Coppock, H.W.: Drug addiction I. Addiction by escape training. J. Am. Pharm. Ass. 45, 788-791 (1956)
3) Nichols, J.R. and Davis, W.M.: Drug addiction II. Variation of addiction. J. Am. Pharm. Ass. 48, 259-262 (1959)
4) Wikler, A., Martin, W.R. and Piscor, F.T.: Factors regulating oral consumption of an opioid (etonicitine) by morphine-addicted rats. Psychopharmacol. 5, 55-76 (1963)
5) Kumar, R., Steinberg, H. and Stolerman, I.P.: Inducing a preference for morphine in rats without premedication. Nature 218, 564-565 (1968)
6) Yanaura, S. and Tagashira, E.: Dependence on and preference for morphine in naive and
7) **YANURA, S. AND TAGASHIRA, E.:** Dependence on and preference for morphine in naive and morphine-experienced rats (II): Comparison of preference formation of morphine, phenobarbital and diazepam in naive and drug dependent rats. *Folia pharmacol. japon.* **71**, 285–294 (1975) (Abs. in English)

8) **YANAGITA, T. AND TAKAHASHI, S.:** Dependence liability of several sedative-hypnotic agents evaluated in monkeys. *J. Pharmacol. exp. Ther.* **185**, 307–316 (1973)

9) **GÖTHESTAM, K.G.:** Intragastric self-administration of medazepam in rats. *Psychopharmacol.* **28**, 87–94 (1973)

10) **SMITH, S.G., WERNER, T.E. AND DAVIS, W.M.:** Technique for intragastric delivery of solutions: Application for self-administration of morphine and alcohol by rats. *Physiol. Psychol.* **3**, 220–224 (1975)

11) **WEEKS, J.R.:** Experimental morphine addiction: Method for automatic intravenous injections in unrestrained rats. *Science* **138**, 143–144 (1962)

12) **HEADLEE, C.P., COPPOCK, H.W. AND NICHOLS, J.R.:** Apparatus and techniques involved in a laboratory method of detecting the addictiveness of drugs. *J. Am. Pharm. Ass.* **44**, 229–231 (1955)

13) **AMIT, Z., BROWN, Z.W. AND SKLAR, L.S.:** Intraventricular self-administration of morphine in naive laboratory rats. *Psychopharmacol.* **48**, 291–294 (1976)

14) **YANURA, S., SUZUKI, T. AND TAGASHIRA, E.:** Study of drug dependence in rats—substitution test and time course of body weight—. *Folia pharmacol. japon.* **70**, 649–658 (1974) (Abs. in English)

15) **YANURA, S., TAGASHIRA, E. AND SUZUKI, T.:** Physical dependence on morphine, phenobarbital and diazepam in rats by drug-admixed food ingestion. *Japan. J. Pharmacol.* **25**, 453–463 (1975)

16) **YANURA, S. AND SUZUKI, T.:** Cross-dependence between phenobarbital and alcohol in rats. *Japan. J. Pharmacol.* **27**, 751–753 (1977)

17) **KAGAWA, M.:** An analysis of alcohol preference in rats (6). Positivity and contingency of alcohol selection and rejection. Presented at the 37th meeting of Japanese Psychological Society (1973)

18) **STOLERMAN, I.P. AND KUMAR, R.:** Preferences for morphine in rats: Validation of an experimental model of dependence. *Psychopharmacol.* **17**, 137–150 (1970)

19) **STOLERMAN, I.P., KUMAR, R. AND STEINBERG, H.:** Development of morphine dependence in rats: Lack of effect of previous ingestion of other drugs. *Psychopharmacol.* **20**, 321–336 (1971)

20) **STOLERMAN, I.P. AND KUMAR, R.:** Regulation of drug and water intake in rats dependent on morphine. *Psychopharmacol.* **26**, 19–28 (1972)