EATING PATTERN OF MORPHINE DEPENDENT RATS

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Abstract—To analyze the drug ingestion patterns of rats in the course of dependence development while on the drug-admixed food (DAF) method, an automatic food intake measuring apparatus was developed. Rats were put on morphine-admixed food, and the food ingestion patterns were recorded with the apparatus in the course of dependence development, during drug withdrawal and at the time of challenge with levallorphan. The naive rats ate the regular diet intermittently at night, and the eating time of morphine-treated rats was longer than that of naive rats. The treated rats also exhibited a frequent eating behavior after 4–5 days on the morphine treatment. During morphine withdrawal, the animal gradually ate the regular diet at about 1-hour intervals, even after evolvement of abstinence signs. When the morphine-dependent rats were given levallorphan, they neither ate nor approached to the food for the first 2–3 hours, but after this time, showed abrupt increases in these activities. Thus, the drug intake pattern of rats in the course of morphine dependence development suggests a correlation between the stage of development of physical dependence and the stage when the animals frequently eat the drug-admixed food.

A quantitative analyzation of abstinence signs in the small animals due to the dependence on morphine-type drugs has often been attempted. Hosoya (1) reported that abrupt weight losses on withdrawal of morphine can be used as an index which can objectively be evaluated. These quantitative studies of physical dependence development have been limited because frequent, repeated injections of high doses of morphine over several weeks were required to render the animals sufficiently dependent (2). From these points 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 (3–8). The morphine pellet method produces morphine-dependent rats or mice in only 3 days. These methods, however, require the subcutaneous implantation of drug reservoirs. The cross-dependence test makes the removal of the pellets or the replacement of drug solutions inevitable. Thus, a method by continuous parenteral infusion has been developed, and the development of dependence on morphine or pethidine by this method has been described (9).

We developed a method of admixing a drug with food (DAF method) and rats could thus be made dependent on narcotic drugs, barbiturates, tranquilizers and alcohol (10–14). This DAF method, unlike the injection method, does not force the experimental animals to ingest a given amount of drug, rather they can eat the drug-admixed food 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 was no apparatus capable of recording of the parameters, we designed automatic equipment by which continuous variations in food intake, eating behavior and approach to food (approach behavior) during the morphine
dependence-inducing period, during withdrawal of morphine, and on application of a narcotic antagonist to morphine-dependent rats could be recorded. The objective was to determine the relationship between the dependence on morphine and the different eating behavior of the animals.

MATERIALS AND METHODS

Development of automatic food intake measuring apparatus (food intakometer): An automatic food intake measuring apparatus (food intakometer) was jointly developed by our department and Natsume Seisakusho, Tokyo. The diagram of the apparatus is shown in Fig. 1. (a) Cage: The cage (21 x 32 x 17 cm) contains 2 chambers, each of which the rats can narrowly enter in symmetrical positions. A photocell is provided at the entrance to each chamber so as to detect the eating behavior of the rat. A feeding hole (1.9 cm in radius) is provided at the end of this chamber opposite its entrance. (b) Balance: A balance for measuring food consumption is situated under each feeding hole. The weighing scale is graduated from 0–10 g, with a minimum graduation of 0.1 g. There are 9 variable ranges with a maximum capacity of 100 g. An electric transducer suitable for indicating the weighing scale is located inside. The design is such that an electric transducing semiconductor (the measuring photocell) is fixed to the balance arm and moves the same as the balance arm. The measuring slit is located to the semiconductor. The compensatory slit and the compensatory photocell have a light source in between. This compensatory photocell is a semiconductor with the same characteristics as the measuring photocell. Variations in

![Diagram of the apparatus](image)

illuminosity of the light source or the effects of temperature on the semiconductor itself are to be offset by the compensatory photocell. Changes in the electric current with changes in the position of the electric transducing semiconductor fixed directly onto the balance arm, (the area illuminated through the slit), are fed into the amplifier. (c) IC amplifier: Changes in the electric current from the electric transducing semiconductor are fed into this 2-channel amplifier and the changes are further subjected to logarithmic conversion, so that they are converted to signals proportional to the movements of the indicator on the scale. The other channel (control) turns off the signals while the animals are eating, is intended to record the eating behavior and is synchronized with the aforementioned photocell. (d) Recorder: The 2-channel signals from the amplifier are fed into the recorder. Because the signals for food intake have already been converted into linear forms in the amplifier, the graduation on the recording chart gives the weight directly. On the other hand, when the animal begins eating, the pen operates in the direction of increment of food intake, and when the animal has finished eating, the pen records any decrease in the amount of food eaten.

When this apparatus was tested by means of decreasing the weights from 10 to 0 g, a linear relationship was achieved between each decrease in the weights and the graduation on the recording chart.

**Eating pattern of morphine dependent rats**

**Experimental animals:** Five-week-old male Sprague-Dawley rats (Charles River Japan, Kanagawa) were used in groups of 6.

**Drugs used:** Morphine hydrochloride and levallorphan tartrate were used. Morphine was applied as an admixture with the usual powder food (CA-1; Japan CLEA, Tokyo) at the concentration of 1 mg/g food.

**Experimental conditions:** The rats were allowed food and drink water *ad libitum*. The lights were turned on at 8:30, and turned off at 18:00. During the experimental period, the animals were raised in a air-conditioned room at a temperature of 22±1 °C and a relative humidity of 55±5%.

**Experimental schedules:** The rats (n=6) were fed the morphine-admixed food (1 mg/g food) for 7 days, the morphine-admixed food was then substituted with the usual laboratory food for 2 days for the purpose of withdrawal. The animals were then again fed the same morphine-admixed food. The food intake, eating behavior and approach behavior during this period were continuously recorded using the food intakometer we designed.

Six other rats were first fed the same morphine-admixed food (1 mg/g food) for 7 days, and then given a narcotic antagonist levallorphan (2 mg/kg) subcutaneously; and their food intake, eating behavior and approach behavior were continuously recorded. The same parameters of naive rats (n=6) as controls were also recorded. During the experimental period, the rats were weighed, and their water intake was measured at 18:00, daily. The food intake was monitored on the recording chart, and the morphine intake was calculated from the readings as shown below.
RESULTS

Figure 2 shows the continuous records of the food intake, and eating and approach behavior of 5-week-old naive rats during the time on the usual diet and also on the 1st day of ingesting the morphine-admixed food. The naive rats had a normal food-eating pattern and most of the eating was done intermittently during the period of from 20:00 to 8:30. The approach behavior was not remarkable. On the other hand, the rats on the morphine-admixed food (1 mg/g food) ate the food frequently at times throughout the day, even from the first day on the diet. Table 1 and Fig. 3 show the food, morphine and water ingestion by naive rats (n=6) and the rats on the morphine-admixed food (n=6) on the 1st day of

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\text{Morphine intake (mg/kg)} = \frac{\text{Morphine-admixed food intake, g/1000}}{\text{Body weight (kg)}}
\]

Fig. 2. Eating patterns of a naive rat (2 graphs in the top half) and those other rats on the 1st day of feeding on a morphine-admixed food (2 graphs in the bottom half). The ordinate denotes the cumulative decreased amount of food, i.e., cumulative food intake (g); and the thick bar on the abscissa, the darkness. Vertical bars on the charts indicate the shutting off of the photocell set at the eating hole, denoting the eating behavior in case the food intake (represented by horizontal lines) is increased, and the approach behavior in case the food intake does not vary. Data on the charts in the top half are those for both food cups containing the usual food; and those on the charts in the bottom half, those for both food cups containing the morphine-admixed food (1 mg/g food). When the intake has reached 10 (g), the lever is adjusted so as to start at 0 (g) again.
morphine treatment. The food intake by naive rats at night accounted for 87.9\% of total food intake per day. On the other hand, the food intake at night by the rats on the 1st day of morphine treatment accounted for 60.3\% of total food intake per day, which was significantly low (P<0.01), compared with the food intake at night by the naive rats.

TABLE 1. Changes in food, morphine and water intake by morphine-treated rats

<table>
<thead>
<tr>
<th>Stage of treatment</th>
<th>Food intake (g)</th>
<th>Morphine intake (mg/kg/day)</th>
<th>Water intake (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Darkness</td>
<td>Light</td>
<td>Total</td>
</tr>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st day</td>
<td>13.6±0.7</td>
<td>2.0±0.4</td>
<td>15.5±0.6</td>
</tr>
<tr>
<td>(87.9±2.1)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>7th day</td>
<td>15.2±1.9</td>
<td>2.5±0.6</td>
<td>17.7±1.6</td>
</tr>
<tr>
<td>(85.7±2.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphine-treated group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st day (dependence)</td>
<td>9.2±1.6</td>
<td>6.0±2.0</td>
<td>15.2±0.8</td>
</tr>
<tr>
<td>(60.3±8.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7th day (withdrawal)</td>
<td>13.5±1.3</td>
<td>3.5±0.7</td>
<td>17.0±1.1</td>
</tr>
<tr>
<td>(79.0±4.1)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>8th day (dependence)</td>
<td>8.7±0.6</td>
<td>2.5±0.5</td>
<td>11.1±0.7</td>
</tr>
<tr>
<td>(78.2±3.6)</td>
<td></td>
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</tr>
<tr>
<td>10th day (re-administration)</td>
<td>14.1±0.8</td>
<td>3.0±0.7</td>
<td>17.2±1.2</td>
</tr>
<tr>
<td>(82.4±2.8)</td>
<td></td>
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</tr>
</tbody>
</table>

Each value represents the mean±S.E. of 6 rats. a: significantly different from the control group (P<0.01). b: significantly different from the 7th day of the morphine-treated group (P<0.05). c: significantly different from the 7th day of the morphine-treated group (P<0.01). Values in parentheses calculated as percent of total food intake.

FIG. 3. Time course of food intake. The ordinate denotes the food intake (g); and the abscissa, time. The thick bar of the abscissa represents the darkness. Each plot is for the mean of 6 animals. ○○○: Naive rats (at 5 weeks of age). ■■■: Rats on the 1st day of feeding on the morphine-admixed food.

morphine treatment. The food intake by naive rats at night accounted for 87.9\% of total food intake per day. On the other hand, the food intake at night by the rats on the 1st day of morphine treatment accounted for 60.3\% of total food intake per day, which was significantly low (P<0.01), compared with the food intake at night by the naive rats.

Figure 4 shows the eating patterns when the rats acquired morphine dependence (12), i.e., the eating pattern on the 7th day of feeding on the morphine-admixed food, and that
The morphine-dependent rats exhibited the major part of their eating behavior during the period of from 20:30 to 11:00, particularly during the 8-hour period of from 3:00 to 11:00. The food intake increased linearly during this 8-hour period, each animal eating about 10 g of the morphine-admixed food. Such a frequent eating behavior was most evident after 4-5 days of feeding on the morphine-admixed food, while the naive rats exhibited no such frequent eating behavior. The morphine-dependent rats exhibited the eating behavior all during the day during the withdrawal of the drug and such was associated with an increased frequency of occurrence of approach behavior, as compared with the same behavior of the naive rats.

Table 1 and Fig. 5 show changes in food, morphine and water intake during morphine dependence and withdrawal. The nocturnal food intake by the morphine-dependent rats made up 79.0% of total food intake per day. The animals showed a markedly decreased food intake 8–16 hours after the withdrawal of the drug, compared with the intake during the 1st day of withdrawal by substitution with the usual food (on the 8th day of feeding).
morphine dependence period. In this instance, the nocturnal food intake was 8.7 g per animal, this being 4.8 g less than the intake during the morphine dependence period. The daytime food intake was 2.5 g per animal, which also was slightly decreased, compared with the daytime intake of 3.5 g per animal during the morphine dependence period. The total food intake and total water intake during morphine withdrawal were decreased by 34.7 ± 6.8 % and 42.4 ± 10.1 %, compared with level during the morphine dependence on the preceding day. The mean weight loss, (an index of abstinence signs) was as marked as 9.2 ± 2.0 % and diarrhea and soft feces also occurred.

After the 2-day withdrawal, the animals were again put on the same morphine-admixed food. They exhibited such a markedly frequent eating behavior and an approach behavior seen during the initial morphine dependence period. The nocturnal food intake was equivalent to 82.4 % of total food intake per day.

Figure 6 shows the time course of morphine-admixed food intake by the morphine-dependent rats and the morphine-dependent rats given 2 mg/kg of levallorphan. Administration of levallorphan to the morphine-dependent rats produced abstinence signs such as loss in body weight and diarrhea in about 30 minutes. In 2–3 hours, the maximum loss in

Fig. 5. Time courses of food intake at morphine dependence and on withdrawal. The ordinate denotes the food intake (g); and the abscissa, hours. The thick bar of the abscissa indicates the period of darkness. Each plot represents the mean of 6 animals. ——: Morphine-dependent rats (after 7 days of feeding on the morphine-admixed food). ——: Rats on morphine withdrawal.

Fig. 6. Time courses of food intake by rats at morphine dependence and by morphine-dependent rats given levallorphan (2 mg/kg, s.c.). The ordinate denotes the food intake (g); and the abscissa, hours. The thick bar of the abscissa shows the darkness. Each plot represents the mean of 6 animals. Levallorphan was administered at 18:00. ——: Morphine-dependent rats given levallorphan. ——: Morphine-dependent rats.
body weight occurred, after which these signs disappeared. When such a phenomenon was considered from the point of time course of food intake, the animals did not eat the food for about 2 hours after levallorphan had been given, however showed abrupt increases in food intake thereafter, the intake 4-12 hours after the application exceeding the intake by the morphine-dependent rats. The intake then abruptly decreased, and tended to be suppressed as a whole, compared with the food intake by the morphine-dependent rats. The nocturnal food intake by the levallorphan-treated animals was 15.5 g per animal, which was 89.0 ±4.3% of the daily total food intake of 17.4±1.7 g per animal. These animals showed neither an eating nor approach behavior for 2-3 hours after the application of the drug, but there was an abrupt increase in frequency which continued for about 9 hours, and then decreased.

**DISCUSSION**

Hill and Stellar (15) in 1951 developed an electric drinkometer for the purpose of continuously measuring water intake by animals. Stolerman and Kumar (16) in 1972 studied the preference of animals for morphine, using this electric drinkometer. Recently, Kurihara et al. (17) developed an apparatus capable of continuously measuring water intake by animals, and studied the effects of hypophysectomy on the circadian water drinking pattern of rats.

There has been, however, no apparatus which could continuously monitor food intake. The automatic food intake measuring apparatus (food intakometer) we designed monitors continuously the intake of food and records eating and approach behavior. This apparatus should prove most applicable in studies of behavioral pharmacology, toxicology and nutrition.

The grade of severity of physical dependence is closely related to the dose, period and frequency of drug application (18). Therefore, it is necessary in experiments of physical dependence development to clarify these factors. Although the DAF method allows rats free access to the drug-admixed food, it does not provide information on the explicit frequency of the drug ingestion. The development our food intakometer enables clarification of the drug intake pattern of animals in the course of physical dependence development.

Naive rats usually eat at night and infrequently during the day. However, when naive rats were fed a morphine-admixed food, they ate the food not only at night but also in the daytime, even on the 1st day on the diet. In other words, the eating behavior of the rats on the morphine-admixed food is increased in frequency, compared with that of naive rats. The effect of morphine on spontaneous locomotor activity varies with the dose. A small dose of this drug exerts an excitatory effect and increases the frequency of eating and drinking behavior (19). A large dose, on the other hand, exerts an inhibitory effect at first, and then an excitatory effect (19). The increased eating behavior of the rats ingesting the morphine-admixed food is apparently due to the excitatory effect of the drug. Furthermore, changes in the eating pattern of the animals may be due to the fact that the food tasted bitter, they could not get enough to supply the daily food requirement from 20:00 to 8:30 hours and they
ate outside these hours.

When the rats continuously ingested the morphine-admixed food hours of food ingestion gradually became close to those seen in naive rats. On the 7th day of feeding on the morphine-admixed food when the animals had acquired an ample dependence on the drug (12), the eating time of the morphine-treated rats was longer than that of naive rats. This is probably due to the fact that when morphine is withdrawn from the dependent rats, weight losses begin to occur from about 8 hours after the withdrawal onwards (10). If the morphine dependent rats, (like naive rats), scarcely eat the food in the daytime, abstinence signs will begin to evolve; hence, the eating time of morphine-dependent rats is prolonged. Furthermore, this shows that the DAF method in which the rats eat the morphine-admixed food frequently rapidly produces morphine-dependent rats. The eating pattern, however, showed the very frequent occurrence of eating and approach behavior from 4-5 days of feeding on the morphine-admixed food onwards. Since such a pattern was not observed in feeding naive rats on the usual diet and also during 1-4 days after the start of feeding rats on the morphine-admixed food, the treatment of rats with morphine by the DAF method for 2-4 days results in the development of physical dependence (20). Thus there is a close relation between the stage of development of physical dependence on morphine and the stage when the animals eat the food frequently.

It has already been reported that abstinence signs such as loss in body weight, decreased food intake, decreased water intake, suppressed spontaneous locomotor activity, diarrhea, etc. are observed (1, 7, 8, 21, 22). These abstinence signs were observed in the present study as well.

The present study revealed that the time courses of eating behavior, approach behavior and food intake of morphine-dependent rats were similar in pattern to those at morphine dependence during the first about 8 hours of withdrawal, but then such were evident at about 1-hour intervals. The evolvement of abstinence sign may explain why these changes were observed from about 8 hours of withdrawal onwards (10). The fact that the eating and approach behaviors occurred at about 1-hour intervals even after the evolvement of abstinence signs may indicate the need of the animals for morphine. Weeks (23) has shown in studies on intravenous self-administration of morphine, that the frequency of lever pressing increased during withdrawal. This lever pressing appears to resemble the eating and approach behavior that occur after the evolvement of abstinence signs.

When morphine-dependent rats were treated with levallorphan, neither the eating nor approach behavior occurred for 2-3 hours. This phenomenon may be interpreted to have been derived from the mechanism that severe abstinence signs abruptly evolved on application of the narcotic antagonist, with the peak abstinence occurring 2-3 hours after the application (12). The precipitated animals then ate voraciously more morphine-admixed food than the morphine-dependent rats. This increased food intake appears to have resulted from the animals trying to rapidly recover from the state of abstinence (19, 20).

The development of an automatic food intake measuring apparatus (food intakometer) has made it possible to record continuously food intake and the eating and approach beha-
viors of the animals. The results of the present study using this apparatus suggested that the dependence on morphine is related to such behavior.

REFERENCES

1) HOSOYA, E.: Some withdrawal symptoms of rats to morphine. Pharmacologist 1, 77 (1959)