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Preferences for Opioids by the Weight Pulling Method in Rats

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SUZUKI, T., Y. MASUKAWA, T. KAWAI AND S. YANAURA. *Preferences for opioids by the weight pulling method in rats.* PHARMACOL BIOCHEM BEHAV **35**(2) 413–418, 1990.—The preference for morphine and codeine was studied by means of the antagonistic conflict behavior 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 drug-admixed food. The experiments began with the preadministration of the drug through the repetition of CFF schedule. The schedule consisted of one choice trial between the intake of normal food and drug-admixed food followed by two consecutive forced trials, in which the rats were forced to take the drug-admixed food only. In the test trial, the findings were that the rats which had already shown a drug seeking behavior toward morphine or codeine pulled weight to take each drug and that the reinforcing effects of these drugs on the drug seeking behavior depended on the treatment period of these drugs. The reinforcing effect of codeine was weaker than one of morphine. It is suggested that the reinforcing effects of these opioids can be evaluated quantitatively by the weight pulling method in rats.

Weight pulling method Morphine Codeine Drug-admixed food Preference Drug seeking behavior Reinforcing effect Choice Conflict test Rats

IF one attempts to define psychic dependence on a drug in terms of desire for intake of the drug, one would run into the problem of how to measure the intensity of such desire. The preference test, in which subjects are allowed a choice between drug and vehicle, does not unequivocally indicate the intensity of desire for the drug. On the other hand, self-administration of a drug by the progressive ratio schedule (15) requires a considerable length of time for a single administration where a high ratio is used, and, thus, may limit the amount of drug intake and possibly give a misleading picture of the drug's reinforcing effects.

We have undertaken a series of observations on the antagonistic conflict behavior resulting from the presence of positive and negative drives in an attempt to devise a simple method of determining the intensity of desire for drug intake using small animal species. The reinforcing effects of several opioids were demonstrated in rats using an oral self-administration method (8). Using a different approach, Brown (2,3) devised an experimental procedure in which animals must pull a certain weight to obtain food, and designed an apparatus to measure how the amount of weight they pull varies with the length of deprivation of food they suffer. We modified his apparatus and used it on those rats which had already shown drug seeking behavior toward morphine or codeine to see how much weight they would pull when weight pulling was compensated by drug delivered by the drug-admixed food method. We also observed the behavioral pattern with the aid of a kimograph (10–12). A method like the weight pulling method which uses conflict behavior as a measure has some inherent problems; for example, there is a possibility that the sedative or stimulating action of the drug may influence the weight pulling behavior. In spite of this, however, an attempt was made here to estimate the reinforcing effects of drugs by means of a behavior different from that prevailing in self-administration procedures using progressive ratio schedules. In the previous study, we estimated the degree of dependence on the drug from a composite of two factors, the concentration of drug administered and the period of drug administration, and related it to the degree of weight pulling. In the present study, the apparatus previously used by us (10–12) was further modified and the degree of dependence on a drug was estimated from the period of drug administration at a constant drug concentration to find a clear effect of drug. The relationship between dependence on the drug and weight pulling was also examined. Morphine and codeine were used as drugs.

METHOD

Animals

Male Sprague-Dawley rats (Tokyo Experimental Animal Ltd., Tokyo) were used in groups of ten. All rats were housed individually in a home cage. Powder food (CA-1; Clea Japan Inc.,

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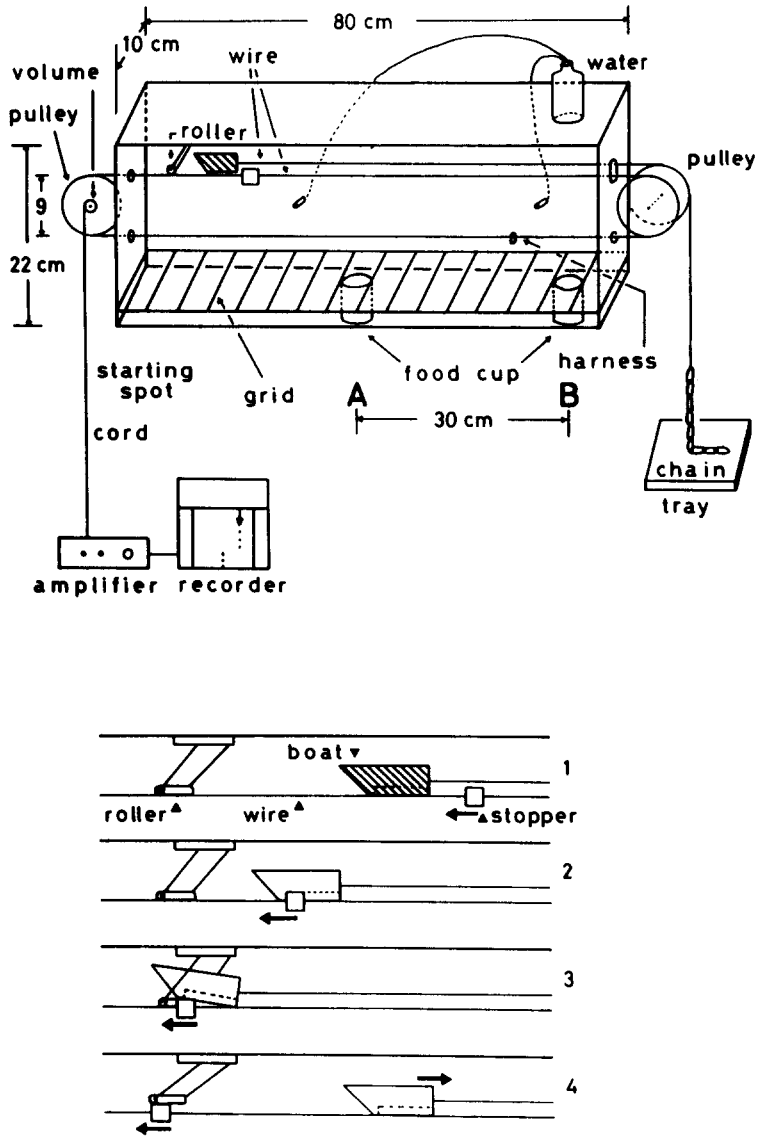

FIG. 1. Schematic diagrams of the apparatus and of the mechanism which releases a loaded weight.

Tokyo) and tap water were supplied ad lib. The animal room was artificially illuminated daily from 8:30 a.m. to 8:30 p.m. and maintained at 21 ± 1°C.

Drugs

Morphine hydrochloride (Sankyo Co., Ltd., Tokyo) and codeine phosphate (Sankyo Co., Ltd., Tokyo) were used. Drug was mixed with powder food for animals at a ratio of 0.5 mg of drug to 1 g of animal food before use (16). The drug-admixed food thus prepared will be referred to as the DAF.

Apparatus

The apparatus is shown in the top half of Fig. 1. It is principally made up of a runway measuring 10 cm in width, 80 cm in length and 22 cm in height. The runway has a floor covered with cross grids and two food cups are placed 30 cm apart on the floor (at point A and B in Fig. 1). Endless wire mounted on pulleys placed outside at opposite ends runs lengthwise through the runway and can be pulled into motion. A stopper and a harness are fixed to the wire. Each rat has a collar put around its neck and the collar is connected with the harness during the experiment. Another length of wire runs in parallel, mounted on another pulley placed outside near point B, and has a chain of links as weight at the outer end and a boat at the inner end.

At the start of the experiment, the rat held at the starting spot is released to move in the runway. The moment the rat passes point A, the stopper catches the boat and pushes it, pulling up the chain and causing a load to strain the rat. As the rat draws nearer and nearer point B, it pulls the weight higher and higher, carrying an increasingly heavy load. The instant the rat reaches point B, the boat pushed by the stopper touches the roller. Upon further advance of the stopper, the stopper catches the roller and sets the boat free (see the bottom half of Fig. 1). When this happens, the rat is released from the load and is able to take the food in the cup

at point B freely. If a rat goes backward from B to A, then turns around and moves toward B again, the same process as above would be repeated: The load on the rat would increase as the rat moves away from point A. This relationship is correlative. Each link of the chain weighs 30 g, and the apparatus, as constructed, allows addition of up to 17 links. To keep the length of wire inside the runway stretched taut, it carries one link at all times. Therefore, the rats pull one link in all the experiments and an additional number of links in the conflict tests to be described later. A potentiometer (Chino, Ltd., Tokyo, EH800-06) records the number of times a rat approaches point B within a given period of time or how long it stays at point B.

Procedure

The experiment is carried out in three steps: preliminary feeding, drug administration and determination of the degree of drug preference. The rats are fed preliminarily in separate cages for a week. The intake of food is controlled in this period by feeding the rats only 6 hours a day, from 10:30 a.m. to 4:30 p.m. Water is given without restriction. A collar is put on each rat in this period. One week later, drug is administered in accordance with the CFF schedule, which comprises one choice trial (abbreviated C) followed by two consecutive forced trials (abbreviated F). In the choice trial, normal food is placed in the cup at point A and the DAF in the cup at point B so that the rat can choose either the normal or drug-admixed food. In the forced trial, the cup at point A is taken out and the DAF is placed in the cup at pont B; hence the rat is forced to take the drug at the time of feeding. This CFF schedule was repeated 2 to 13 times. Three groups, G_2,G_4 and G_6 were given morphine, whereas five groups, G_2, G_4, G_6, G_9 and G_{13}, were given codeine. Here, the subscript designates the number of repetitions of the CFF schedule. After completion of the experiment using the CFF schedule, each group was subjected to four consecutive choice trials.

Up until this point, only a single link of chain is attached to the wire (to insure that it remains taut). Then, to see how the preference for drug varies following the preceding period of drug administration, test trials were conducted by means of a conflict test. The conflict test conditions are as follows. Normal food is placed in the cup at point A and the DAF in the cup at point B. The moment the rat passes point A in its move toward point B, the weight of chain links begins to strain its neck and, unless the rat pulls this weight with it, it cannot reach point B and, hence, cannot take the DAF. The load on the rat starts at 5 links and increases by 2 links at a time until the rat abandons its weight pulling behavior to reach point B. The test was repeated twice for each number of links. The apparatus is constructed in such a manner as to allow addition of up to 17 links.

All the experiments were carried out, one trial a day, between 10:30 a.m. and 4:30 p.m. After completion of the experiment for the day, the rats were returned to their home cages and given only water. After each trial, the normal food intake and the DAF intake were determined and the preference rate was calculated as follows:

$$\text{Preference rate (\%)} = \frac{\text{DAF intake (g)}}{\text{Normal food intake (g)} + \text{DAF intake (g)}} \times 100$$

The rats were weighed before and after the experiment. Additionally, the number of rats that reached point B was counted and the frequency of the approach behavior toward point B shown by each rat in the 6-hour period from 10:30 a.m. to 4:30 p.m. was recorded in the conflict test trials.

The control groups, one for the morphine test groups and another for the codeine test groups, were not subjected to the experiments by the CFF schedule, that is, they were not given morphine or codeine. They were preliminarily fed for a week in the normal manner and then trained for running inside the runway and locating point A and B the next two days. In this training, the normal food was placed in both food cups at A and B. Thereafter, like the test groups, the control groups were subjected to four consecutive choice trials and then to the conflict test. As the period of drug administration differs from group to group, the rats assigned to each group were chosen in respect to age such that all the rats in any group became 13 weeks old at the start of the conflict test.

Statistical Analysis

The proportion of the total number of rats in each group that reached point B pulling a given number of links and obtained DAF was calculated. The results of these calculations were transformed according to an inverse sine function, and were thereafter subjected to an analysis of variance (ANOVA).

RESULTS

The preference rate for morphine-admixed food in the choice trials is shown in Fig. 2 and that for codeine-admixed food in Fig. 3 (G_2, G_4 and G_6 groups) and Fig. 4 (G_6 G_9 and G_{13} groups). Each point in these figures represents the mean of six rats. With either morphine or codeine, the preference rate rose gradually as the CFF schedule was repeated during the first experiment. On the other hand, each control group which had not been given the drug showed a preference rate of practically zero, except on the first day, in the 4-day period of daily choice trials. The preference rate of about 20% for both control groups on the first day may be attributed to the after effect of the training conducted on the preceding days for running in the runway and locating the food cups at point A and B.

The conflict test involving weight pulling is conducted, as described in the Procedure section, starting at a weight of 5 links (150 g), adding 2 links (60 g) at a time thereafter, and repeating the conflict test twice for a given weight. The maximum number of links was 11 for the morphine groups and 17 for the codeine groups. The proportion of rats in each group that reached point B pulling a given number of links is shown for the morphine groups in Fig. 5 and the codeine groups in Fig. 6. As no systematic differences were present in the tests repeated twice for a given weight, the total number of rats ($n=12$) was obtained by multiplying the number of rats ($n=6$) with the number of repetitions (2 consecutive days). The notation of one link (30 g) in these figures refers to the 4-day period of daily choice trials (see the Apparatus section), and each point for one link represents the mean of 4 days. The rats in the control groups for both drugs stopped moving toward point B the instant the weight was increased, and the addition of links was therefore stopped at 5 (150 g). As is seen from Fig. 5, for the group given morphine, the number of rats taking the DAF at each weight was greater at each successively longer period of drug administration by the CFF schedule.

The effect of the length of the morphine administration period was significant, $F(2,8)=20.63$, $p<0.01$. On the other hand, codeine produced this relationship only after a longer period of drug administration than morphine. In the groups treated with codeine for a short period comparable to the morphine-treated groups, namely the G_2, G_4 and G_6 groups, the proportion of the total number of rats in a group that took DAF did not depend on the length of the CFF administration period. In G_6, G_7, G_9 and G_{13} groups, however, significant effects were shown, $F(3,12)=5.72$, $p<0.05$. Furthermore, after the same period of drug administration, for G_4 and G_6, a higher proportion of animals that received morphine in the CFF procedure obtained DAF in the conflict

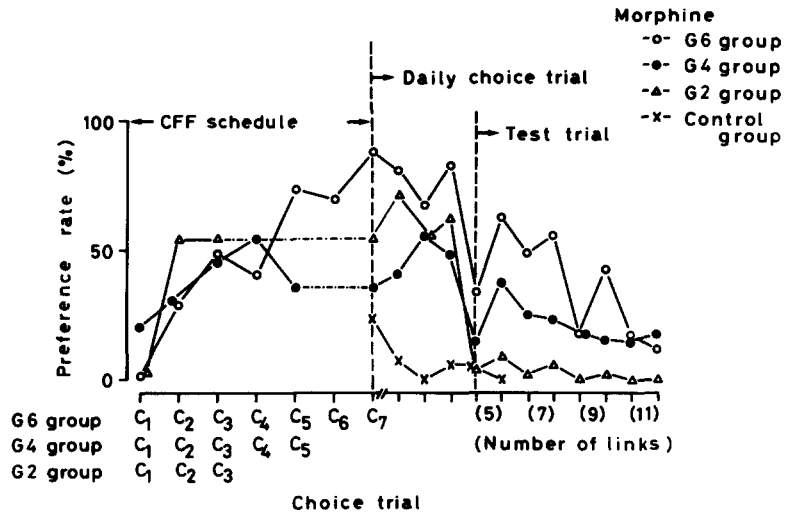

FIG. 2. Preference rate for morphine-admixed food in choice trials and test trials. Each plot represents the mean of 6 rats.

procedure than animals that received codeine. The data for preference rate (percentage of total food obtained as DAF) showed tendencies similar to those seen in the proportion of animals in each group taking DAF.

There was virtually no effect of added weight on the total food intake in any of the groups, and a normal increase in the body weight was found in the tests extending from three to ten weeks.

DISCUSSION

Experimentally quantifying the reinforcing effects of drugs can be difficult. Beach (1), Nichols *et al.* (9), Kumar *et al.* (7), Yanaura and Tagashira (17), Yanaura and Suzuki (18) and Yanaura *et al.* (19) studied the preferences for drugs of small animals and Yanagita (15) applied the progressive ratio method of drug self-administration to monkeys with good results. The method used in the present study differed slightly from methods we have previously used, and attempted a more quantitative assessment of drug-seeking by using behavior in a conflict situation as a major dependent variable. We have previously suggested that the amount of weight pulled in the process of obtaining access to morphine and codeine might indicate the degree to which these drugs serve as reinforcers (10,11). The present study aimed at obtaining a more quantitative assessment of drug-seeking behavior with respect to these two drugs.

As mentioned in the Introduction, drug concentration was kept constant for all test groups in the present study; the major independent variable was the period of time that subjects were allowed to self-administer a drug in the CFF procedure. In the control group, which received no pretreatment with morphine, subjects did not travel to point B (where DAF was available) when weight was added to the chain. Moreover, the number of mor-

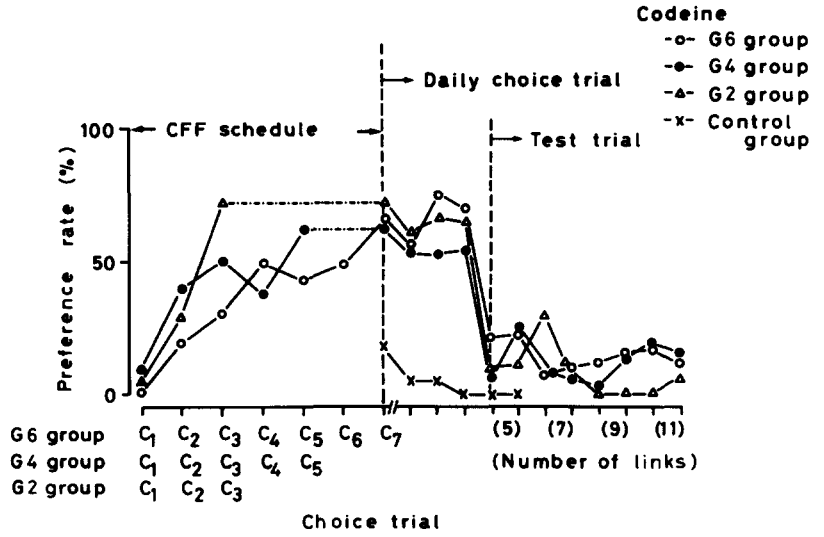

FIG. 3. Preference rate for codeine-admixed food in choice trials and test trials in G_2, G_4 and G_6 groups. Each plot represents the mean of 6 rats.

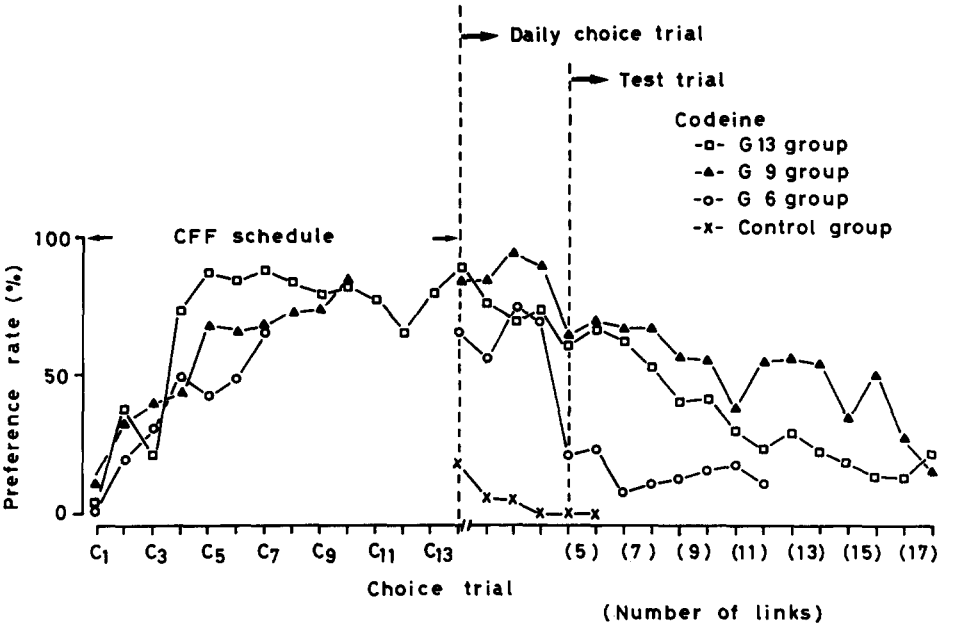

FIG. 4. Preference rate for codeine-admixed food in choice trails and test trials in G_6, G_9 and G_{13} groups. Each plot represents the mean of 6 rats.

phine-trained rats that took DAF at point B at each weight value was directly related to the length of the period in which subjects were initially allowed access to morphine in the CFF procedure. These results suggest that the amount of weight that rats will pull can be seen as a valid measure of the reinforcing effects of morphine, for rats which have been exposed to morphine self-administration for varying lengths of time. Weeks (14) reported that the reinforcing effects of morphine were intensified by withdrawal of morphine (which resulted in the appearance of withdrawal signs), which indicates that morphine possibly also can reinforce drug-taking behavior by suppressing withdrawal signs resulting from physical dependence on morphine. It was shown by Deneau and Seevers (5) that the degree of physical dependence is affected by three factors, namely dose of the drug, frequency of treatment and treatment duration. Yanagita (15) reported that for monkeys responding under a progressive ratio schedule, the

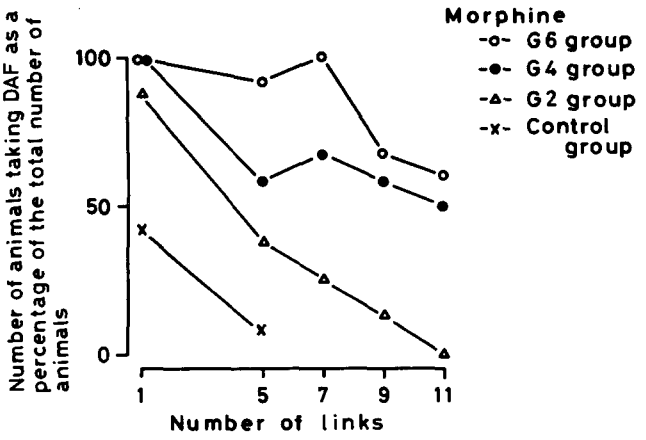

FIG. 5. The relation of the percentage of animals taking morphine-admixed food against the number of chain links.

breaking point for morphine was increased by morphine pretreatment. We also reported previously (10) that the amount of weight rats would pull was greatly increased when they were made severely physically dependent on morphine by manipulating drug concentration and the period of drug administration. It is possible that in the present study the reinforcing effectiveness of morphine, which was directly related to the duration of exposure to administration under CFF conditions, may have been determined not solely by its positive reinforcing effects, but also by its termination of withdrawal signs caused by the presence of physical dependence. Although we did not test for this, the rats in the present study may indeed have been physically dependent on morphine, considering that in a previous study (13) which used the same morphine admixed food (0.5 mg/g of food) and the same CFF schedule as was used in the present study, physical dependence on morphine was produced, as well as a gradual increase in the preference rate for morphine admixed food.

The reinforcing effectiveness of codeine may also have resulted both from codeine's positive reinforcing effects and its ability to reinforce drug-traking behavior though termination of withdrawal signs, because codeine's reinforcing effects, also generally were directly related to the length of administration under the CFF procedure. Although it has previously been reported that codeine is absorbed better than morphine via the oral route of administration (6), in the present study the reinforcing effects of codeine were weaker than those of morphine after the same period of drug administration for both the G_4 and G_6 groups, and a longer treatment period was required for codeine to produce reinforcing effects similar to those of morphine.

The above findings demonstrate that the reinforcing effects of morphine and codeine depend on the length of the treatment period with these drugs. These results also suggest that morphine is a better reinforcer than codeine in the range of drug concentrations used in the present study, which agrees with results obtained using the intravenous route of self-administration (4). The present study demonstrated that the weight pulling method can be used to evaluate quantitatively the reinforcing effects of these drugs,

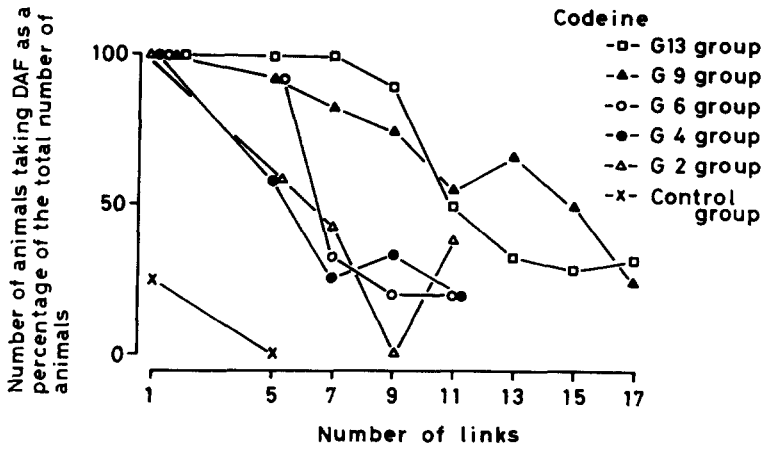

FIG. 6. The relation of the percentage of animals taking codeine-admixed food against the number of chain links.

whose reinforcing effects may be due both to their positive reinforcing effects and to their ability to terminate withdrawal signs. It will be necessary to use the current procedure to study the reinforcing effects of a drug such as cocaine, which does not produce physical dependence, to prove that the procedure can be used to qualitatively and quantitatively evaluate the positive reinforcing effects of drugs that do not also simultaneously reinforce behavior by terminating withdrawal signs.

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