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After Two Weeks Habituation to Capsaicinized Food, Rats Prefer This to Plain Food

BASSAM DIB[1]

Université Claude Bernard, Laboratoire de Physiologie A, Faculté de Médecine Grange Blanche 8 avenue Rockefeller, F 69373 Lyon CEDEX 08, France

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DIB, B. *After two weeks habituation to capsaicinized food, rats prefer this to plain food.* PHARMACOL BIOCHEM BEHAV **37**(4) 649–653, 1990.—In a first series of experiments rats were accustomed for two weeks to eat chow with capsaicin (250 μg: 1 g of food). After this habituation period, when free to choose, 3 rats out of 4 preferred eating the piquant chow. In a second series of experiments the rats had access to a choice of chows without any habituation period. In this series, the choice between piquant and nonpiquant chow showed that 4 rats out of 5 preferred to eat the chow without capsaicin. The present experiment shows that the piquant chow eaten by rats produced a fall in rectal temperature 48 h later. The present experiment provides some evidence that rats accustomed to eat piquant food manifested, when free to choose, a preference for an innately unpalatable piquant chow.

Chow Capsaicinized chow Hypothermia Behaviour

CAPSAICIN (8-methyl N-vanillyl-6-nonenamide) is the parent molecule of a number of structurally related compounds which give a variety of red peppers their pungent flavour (21). This substance affects a range of physiological processes stretching far beyond those involved in creating its gustatory appeal. The pharmacological study of capsaicin can be traced back over more than a century to when Högyes (11) found hypothermia in the dog after intragastric administration of an oily extract of paprika (Hungarian red pepper).

The most incisive early contributions were made by Jancso and his wife, Jancso-Gabor, through work they began in the late 1940s and continued for the two subsequent decades. These authors demonstrated that capsaicin affects circulation, respiration, the sensory system and temperature regulation. The remarkable discovery was that the immediate effects of acute low doses were distinguishable from the long-lasting delayed effects after chronic doses of capsaicin (14). Both the acute and chronic pharmacological effects of capsaicin on thermoregulation have been studied (2, 5–7, 12, 15).

Capsaicin is the active substance responsible for the irritating and pungent effects of various species of hot peppers used as food additives. Chili peppers and black pepper are each consumed daily by hundreds of millions of humans. Humans frequently develop a liking for innately unpalatable bitter or irritant substances, while this occurs very rarely in nonhumans (4,22). Preference for the pungent flavour of chili pepper involves an affective shift when an innately disliked sensation becomes liked (18). There is no physiological explanation for this phenomenon. As regards animals, rats failed to come to like chili pepper (10,12). In contrast, acquired preferences for piquant food by chimpanzees have been observed (19). In this study an attempt is made to establish an animal model (laboratory rats) for the development of preference for an innately unpalatable and capsaicin-adulterated food.

METHOD

Thirteen OFA male rats weighing 250–260 g were used. The animals were housed in individual cages under a 12-h light/12-h dark cycle in a temperature-controlled room at 22°C. The rats were divided into two groups: Group 1: n = 8 rats; Group 2: n = 5 rats.

Experiment I was conducted on group 1 (n = 8 rats). They were accustomed for two weeks to eat chow mixed with capsaicin (250 μg: 1 g of food). After the habituation period, 4 of these rats were placed in a situation of choice between plain chow and chow with a low dose of capsaicin (250 μg: 1 g of food). In contrast, the other 4 rats were given chow with a high dose of capsaicin (1,000 μg: 1 g of food). In this case, the 4 rats refused to eat the chow with a high dose of capsaicin, and the experiment in this subgroup was stopped two days later.

Experiment II was conducted on group 2 (n = 5 rats). In this group the choice between plain chow and chow with capsaicin (250 μg: 1 g of food) was offered immediately, without any train-

[1]Requests for reprints should be addressed to Dr. B. Dib, Center for Studies in Behavioral Neurobiology, Concordia University, 1455 de Maisonneuve Blvd. W., Montreal, Quebec, Canada H3G 1M8.

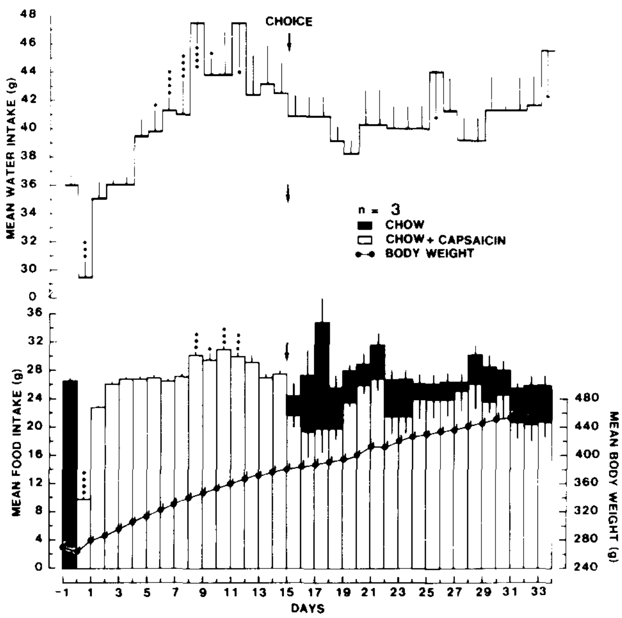

FIG. 1. Mean (±SEM) water intake (g) (upper figure), food intake and body weight (g) (lower figure), before, during the training period and during the period of freedom of choice. The black circles indicate the body weight evolution on the figure, asterisks indicate a significant variation in water and food intake. When taken before, during and after habituation period (*$p<0.05$, **$p<0.02$, ***$p<0.001$). n indicates the numbers of rats. When they had the choice between piquant and nonpiquant food the rats continued to eat much more piquant food than plain food. Student's t-test shows a significant difference from the first day of the period of freedom of choice till the end of the experiment except on the 17th and 18th days ($p<0.01$, $p<0.001$).

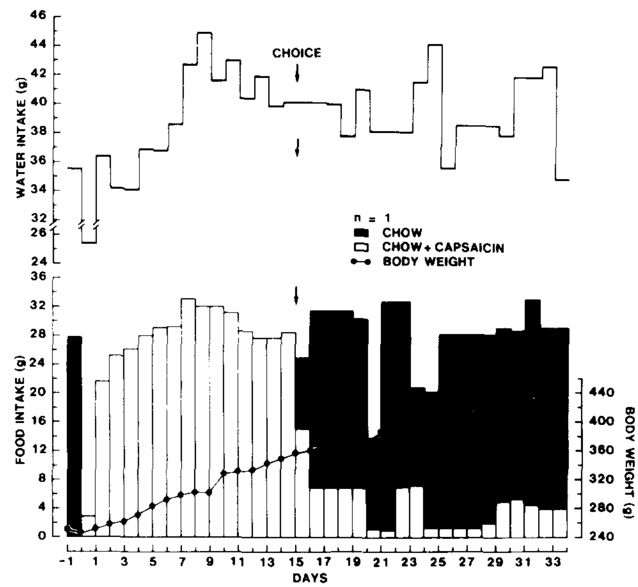

FIG. 2. This figure shows the evolution of water (g) (upper figure), body weight and food intake (g) (lower figure) for one rat which preferred to eat nonpiquant food.

ing period. The chow pellets were ground, capsaicin powder added and the chow stirred with water for one hour in order to obtain a paste. As a control, similar food, but without capsaicin, was prepared. The pastes were dried in a drying oven at 75–80°C.

One week after the drying period, the piquant and nonpiquant food was cut into small pieces, 2×3 cm, and put separately into the rats' hoppers. In the two groups of rats, food intake, body weight and water intake were measured daily in the morning. Dry food and water were available 24 h a day.

In both Experiments I and II, the same preparation of the chow was offered the rats in a situation of choice. During the habituation period, body temperature was measured before, and 48 h after, the rats were given piquant food.

Rectal temperature was recorded using a thermocouple in the colon, 6.5 cm from the anus (Tre). The rats were placed for 5 min in the climatic chamber at an ambient temperature of 22°C.

Statistical analysis was carried out using Student's t-test.

RESULTS

Experiment I: Choice of Food After a Training Period of 15 Days

Figure 1 shows the mean food and water intake and body weight in 3 out of 4 rats before and during the training period and when there was freedom of choice.

The mean food intake one day before the rats were given capsaicinized food was 26.7±0.6 g. When first given capsaicinized chow, their food intake decreased sharply and significantly from 26.7±0.6 to 9.9±0.9 g (Student's t-test, $p<0.001$). On the second day, the rats ate as much capsaicinized food as normal food. The mean intake of capsaicinized food varied between 23.8±0.3 and 31.8±1.3 g. We observed that during the habituation period, food intake increased significantly at the 9th, 10th, 11th, and 12th days from 26.7±0.6 to 37.8±0.8 g as compared with the prehabituation period (Student's t-test, $0.01<p<0.05$). It is important to note that when they had the choice between plain chow and capsaicinized chow, these 3 rats continued to eat much more capsaicinized chow than plain chow.

After the choice between piquant and nonpiquant food was given, the mean decrease in food intake per 24 h (days 15–16) was 21.6±1.6 g. This decrease in food was statistically significant when compared with prechoice intake of piquant food (Student's t-test, $p<0.01$). This decrease in food intake may be due to the conflict behaviour effect resulting from the freedom to choose between the two chows.

The mean food intake under conditions of freedom of choice varied between 19.3±3.2 and 26.8±2.4 g for piquant food, and between 2.5±2.01 and 14.8±3.2 g for nonpiquant food. Student's t-test showed a significant difference between piquant and nonpiquant food from the first day of free conditions of choice of food till the end of the experiment, except on the 17th and 18th days ($p<0.01$, $p<0.001$). Of the 4 rats used under conditions of free choice, one preferred to eat chow without capsaicin (Fig. 2). Its daily food intake varied from 7.9 to 29.7 g for chow alone, and from 1 to 11.2 g for chow with capsaicin.

At the end of Experiment I, the rats that preferred to eat chow with a low dose of capsaicin were again used for the experiment, this time to eat chow with a high dose of capsaicin (1000 μg: 1 g of food). These animals, as the 4 other rats, refused to eat the chow with a high dose of capsaicin, and the experiment was stopped two days later.

Figure 1 also shows mean water intake before and during the training period and the period of free choice. Mean water intake one day before the rats were given capsaicinized chow was 36.2±1

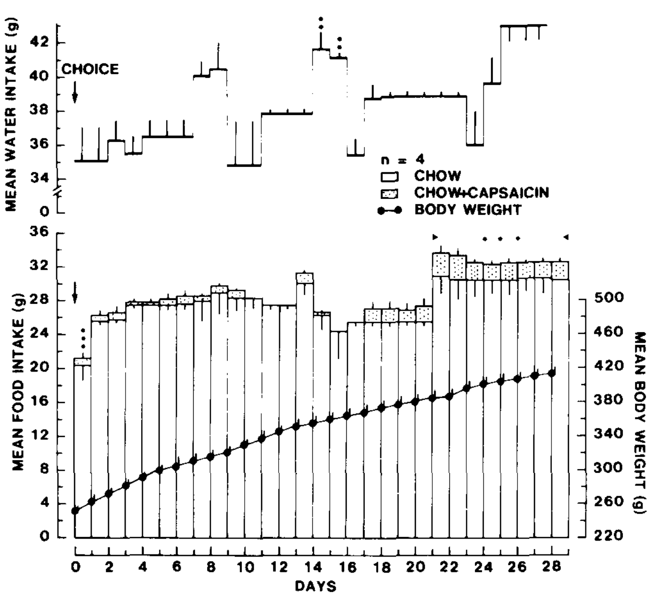

FIG. 3. Mean (±SEM) water intake (g) (upper figure), body weight and food intake (g) (lower figure), during the period of free choice between piquant and nonpiquant chow. The asterisk indicates the level of significance of differences in total food intake between the 1st and 2nd days and between the 21st day and the 22nd, 23rd, 24th, 25th, 26th, 27th and 28th days (***$p<0.01$). The asterisks also show differences in water intake between the 14th day and 15th and 16th day (**$p<0.02$).

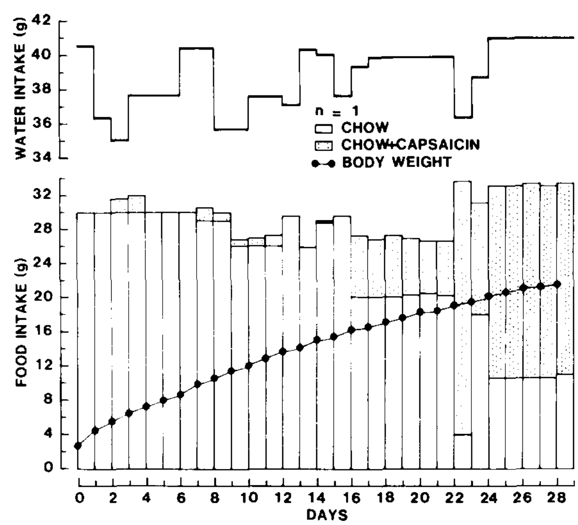

FIG. 4. Shows the evolution of food and water intake, and body weight for one rat which preferred to eat piquant food from the 22nd day till the end of the experiment.

g. This figure shows that in all rats the mean water intake one day after the rats were given chow with capsaicin decreased significantly from 36.2±1 to 29.5±1.2 g (Student's *t*-test, $p<0.01$). However, after an initial decrease in water intake, the rats increased their intake of water. Mean water intake varied between 29.5±1.2 and 47.6±2.9 g. During the training period of free choice, water intake increased significantly in comparison with the pretraining period. Student's *t*-test showed a significant difference on the 6th ($p<0.05$), 7th, 8th, 9th ($p<0.001$), 10th, 11th, 12th, 26th, 33rd and 34th days ($p<0.05$).

Figure 1 shows the mean body weight before and during the training period, and during the period of free choice. The mean body weight in the rats of Experiment I (n=3) was compared to the mean body weight of the rats of Experiment II (n=4). A Student's *t*-test day per day did not show any significant difference.

Figure 2 shows the evolution of food and water intake and body weight for one rat. After the habituation period this rat preferred to eat nonpiquant food. The food intake under conditions of freedom of choice varied between 0 and 11.2 g for piquant food, and between 7.9 and 29.7 g for nonpiquant food. Water intake decreased from the 34th day till the end of the experiment.

Experiment II: Choice Between Piquant and Nonpiquant Chow Without a Habituation Period

The second experiment was conducted to see whether the rats would begin to eat the piquant chow 250 μg: 1 g of food immediately when placed, without any preliminary habituation period, under conditions of free choice, with the same control food as in the first experiment.

Figure 3 presents the mean food and water intake and body weight in 4 rats out of 5 that did not eat the piquant chow. This figure shows that during the experiment the intake of food without capsaicin varied between 25.4±1.5 and 29.9±1.3 g and the intake of piquant chow between 0 and 2.7±1.08 g. We have observed that 1 rat out of 5 began to eat capsaicinized food 3 days after the rats were placed under conditions of free choice of food. From the 17th day till the end of the experiment, the one rat in question ate between 6.3 and 21.8 g of piquant food, as shown in Fig. 4.

The mean quantity (20.4±1.5 g) without capsaicin eaten by the rats from 0–1 day of the experiment was smaller than the mean quantity (25.6±0.5 g) eaten on the second and following days of the experiment. This difference in food intake is statistically significant (Student's *t*-test, $p<0.01$). The decrease in food intake on the first day is perhaps due to the conflict behaviour effect resulting from the freedom to choose between the two chows.

It is important to note that from the 22nd to the 28th day intake of food with and without capsaicin was greater than the intake of piquant and nonpiquant chow from 0 to 21 days. From the 1st to the 21st day the rats ate between 21.2±1.05 g and 30.8±1.2 g piquant and nonpiquant chow. From the 22nd to the 28th day the rats ate between 32.5±0.8 and 33.4±1.6 g piquant and nonpiquant chow. The mean piquant plus nonpiquant food intakes are compared between the 21st, 22nd, 23rd, 24th, 25th, 26th, 27th and 28th days (Student's *t*-test shows a significant difference, $p<0.01$). The small quantity of piquant food eaten by the rats between the 22nd and 28th days may have produced an activation of ingestive behaviour.

Figure 3 also shows mean water intake under conditions of free choice of chow. Mean water intake from the first day of the experiment was 35.2±2.1 g. The mean water intake in all rats varied from 35.2±2.1 to 42.8±1 g. Student's *t*-test showed a significant increase in water intake on the 15th and 16th days, when compared to the first day of the experiment ($p<0.02$). It is quite possible that even the small amount of capsaicinized food ingested produced an increase in water intake.

Figure 4 shows the evolution of food and water intake and body weight for one rat. This rat preferred to eat piquant food, as shown in Fig. 4. Water intake varied between 35.6 and 42.5 g.

Rectal Temperature

Rectal temperature (Tre) was measured before, and 48 h after, the rats ($n=8$) were given piquant chow in Experiment I. Mean Tre before the experiment was 37 ± 0.4°C and mean Tre 48 h after the rats were given piquant chow was 36.4 ± 0.6°C. Student's *t*-test showed a significant difference ($p<0.01$).

DISCUSSION

The present investigation provides some evidence that under conditions of free choice rats accustomed to eating chow with capsaicin preferred this chow rather than noncapsaicinized chow. The rats manifested a preference for innately unpalatable piquant food. This study confirms the previous reports obtained in chimpanzees for their preferences for piquant foods (19). In humans, the majority of adults in the world ingest, every day, several normally rejected substances. These substances often taste bitter or irritate the oral mucosa. They include items of major commercial and/or medical importance, such as coffee, beer, spirits, tobacco, chili pepper, all of which produce an affective shift from dislike to liking (16).

The present experiment confirms the knowledge obtained in humans that naive rats largely refuse to eat piquant food. It is clear that one day after the rats were given piquant "unpalatable" food, the piquant food and water intake decreased in parallel. This initial aversion may be due to capsaicin-induced irritation. Furthermore, one may speculate that the decrease in food and water intake due to the conflict behaviour effect resulting from the freedom to choose between the piquant and nonpiquant food produced an aversion. On the second day of the experiment, during the habituation period, the rats ate the innately unpalatable piquant food and continued to do so in the next new situation of choice between plain chow and piquant food. Rozin et al. (19) argue that the search for causes of acquired preferences for foods is hampered by the absence of a good animal model. For them, it has been very difficult to establish strong stable preferences for foods (taste) in rats. Long exposure to some strong (irritant) spices did not significantly enhance the preference of rats (10). In contrast, chimpanzees and rhesus monkeys acquired a preference for chili pepper (9). More recently acquired preferences for piquant foods by chimpanzees have also been reported (19). These animals are phylogenetically close to man, and were all raised more or less as pets, with close relations to humans. In contrast, the rats that failed to come to like chili pepper (17) were fed with rat chow. In other experiments, Booth et al. (1) have reported enhanced preferences in animals and humans for flavours associated with rapid satiation in hungry subjects. Cabanac and Johnson (3) demonstrated that rats were motivated to leave a thermoneutral refuge and run along a 16-m long labyrinth at an ambient temperature of −15°C to eat highly palatable food rather than chow at home.

The present experiment shows that the piquant food eaten by rats produces a fall in rectal temperature. This is most likely due to vasodilatation. It is possible that the peppers, chiefly used as food additives in warm countries, produce a heat loss by vasodilatation, with a decrease of body temperature. The decrease of body temperature was observed after a capsaicin injection, centrally or peripherally (5–8). In the present experiment, the decrease of body temperature 48 h after the rats were given to eat the piquant chow is due to capsaicin effect. Stary (20) reported that the oral administration of pungent extracts of pepper, black pepper lowered the body temperature of the rabbit. This response was attributed to stimulation of warm-sensitive fibers in the alimentary tract. One can hypothesize that with each meal of piquant food, the piquancy may produce a decrease of rectal temperature with cutaneous vasodilatation. For subjects living in a hot environment, this decrease might be considered as a reward. Therefore, it was possible that there is a relationship between the change of rectal temperature and the activation of ingestive behaviour.

In the present experiment, the preference for piquant food after a habituation period suggests that capsaicin added to food may also facilitate the ingestion and swallowing of food, and may enhance its palatability. The idea that capsaicin facilitates ingestion was observed at the end of Experiment II. Mean daily intake of piquant plus nonpiquant food in Experiment II was greater than intake of food alone (Fig. 3).

The preference for piquant food obtained in Experiment I was acquired during habituation. In the second experiment, though rats were given chow from the same preparation as in the Experiment I, when the choice was given between piquant and nonpiquant food without any habituation period, 4 rats out of 5 preferred to eat the nonpiquant food.

Furthermore, in Experiment I, the rats which preferred to eat piquant food prepared with low dose of capsaicin refused to eat the food prepared with high doses of capsaicin. This may indicate that the quantity of piquant food eaten during habituation (and during the period of conditions of choice) did not abolish gustative receptors. It seems that the rats were able to distinguish between food with low and high doses of capsaicin when the high or low piquant foods were offered separately.

In conclusion, for the present study, acquired preferences for food prepared with low doses of capsaicin was observed in 3 out of 4 rats accustomed to eat the piquant food. The present experiment, though conducted over a restricted number of animals, shows that a preferring for innately unpalatable piquant or irritant food can be obtained in the rat.

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