Various Aspects of Feeding Behavior Can Be Partially Dissociated in the Rat by the Incentive Properties of Food and the Physiological State

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The authors investigated the role of food incentive properties and homeostatic state on the motivational, anticipatory, and consummatory aspects of feeding. Behavioral tests were carried out on food-sated and food-restricted rats that were presented with 2 kinds of food differing in their palatability level. Both food-sated and food-restricted rats consumed large quantities and were highly motivated when presented with very palatable food. In contrast, only food-restricted rats developed anticipatory responses, regardless of the kind of food presented. These data suggest that food incentive properties play a key role in the control of consummatory and motivational components of feeding but seem less involved in the regulation of anticipatory behavior.

Keywords: food intake, motivation, anticipatory activity, palatability, homeostatic state

A critical challenge for neurosciences is to understand the regulation of food intake in mammals (Berthoud, 2004; Kelley, 2004; Saper, Chou, & Elmquist, 2002; Schwartz, Woods, Porte, Seeley, & Baskin, 2000). Feeding is a very complex behavior, which results from the interaction between a wide range of external and internal factors. External factors, including the environment, the season, the time of day, the availability and the quality of food, and the level of stress of the organism, notably affect food intake. For example, it has been shown that environmental cues associated with food delivery can later potentiate eating (Weingarten, 1983; Zamble, 1973). Stressors also have been shown to increase or decrease feeding behavior depending on their magnitude and duration, the quality of the presented food, and the physiological state of the subjects (Burlet, 1988; Howell et al., 1999; Levin, Richard, Michel, & Serviatius, 2000). Similarly, internal factors such as circulating levels of glucose, insulin, leptin, catecholamines, and other molecules modify food intake (Baskin, Wilcox, Figlewicz, & Dorsa, 1988; Saper et al., 2002; Schwartz et al., 2000). The convergence of these interoceptive signals and the exteroceptive properties of the food (i.e., presence, quality) on specific brain areas, participate in the initiation and control of feeding (Orsini, 2003).

The central nervous system properly adjusts food intake in response to the external and internal factors to maintain steady states of energy and nutrient balances (Saper et al., 2002; Schwartz et al., 2000).

We focused our study on the role of two factors: one internal and the other external. Homeostatic state of the animals (food-sated vs. food-restricted) is an internal variable that ensures differential level of drive toward food. The other factor (food incentive properties or palatability, inferred from the amount eaten by the rats when presented with a choice) is a function of the organoleptic characteristics of an aliment but also depends on the motivational state of the subject (Berridge, 1991; Cabanac, 1988). In humans, increased palatability leads to an increase in food intake (Bellisle & Le Magnen, 1980; Bellisle, Lucas, Amrani, & Le Magnen, 1984; Hetherington & Macdiarmid, 1995; Yeomans, 1996).

Feeding is a motivated behavior, which is triggered and maintained when the central nervous system is aroused by specific stimuli (e.g., alimentary; Orsini, 2003). Motivation is characterized by processes dependent on the internal state of the organism, which initiate a subset of specific actions or responses. These processes determine the probability and vigor of a given response (Bindra, 1968). Every motivated behavior can be differentiated into two components or phases: the anticipatory component, also called appetitive or preparatory, is aimed at reward obtainment. It includes exploration, approach, and instrumental response. The consummatory component leads to the final consumption of the reward and includes behaviors such as eating, drinking, and copulation. The experimental dissociation of these components is important as anticipation and consumption are not likely to be affected to the same degree by the internal and external factors mentioned above.

Early studies have focused on the role of either reward magnitude or intensity of the hunger drive on the resulting behavioral outcome. For example, it has been shown that latency to obtain food reinforcement is increased when food is available before...
testing and, conversely, is decreased when the duration of food deprivation increases (Kimble, 1951). The complexity and novelty of a given environment can also decrease the latency to obtain food on a runway when animals are slightly food deprived, indicating that the observed performance is the result of concurrent and multiple variables (Timberlake & Birch, 1967). Notably, the topography of operant behavior is not modified in response to changes in motivation. Rats will increase response frequency with increased food or water deprivation as well as with increased incentive qualities of the reward, but the recorded response topography will remain almost constant (Corman & Miles, 1966). The aforementioned studies tested animals that were either food deprived or sated or that were presented with more incentive rewards while food deprived. In this study, unlike most others, we have measured multiple components of feeding behavior as a function of deprivation level and palatability of the available food at the same time. We have found that anticipatory activity is mainly regulated by food restriction, whereas consumption and running for food are both under the influence of the homeostatic state and food incentive properties.

Method

Animals

Male Wistar rats (Ifa-Credo, France) were used in this study. Groups of 4 rats (weighting 225–250 g when arriving at the laboratory) were housed in clear plastic cages in an animal vivarium maintained on a reversed 12-hr light–dark cycle (lights off at 9:00 am) and at a constant temperature of 23 °C. Following 1 week of ad libitum access to food, half of the animals were placed on a restricted food regimen designed to reduce their body weight to 85% of free-feeding values. Once the animals reached the required weight, they were given 17 g of laboratory chow, generally in the afternoon (late part of dark phase) but never at the same time each day. During experimental sessions the amount eaten by the rats was discounted from the daily ration. In general, 5–7 days after placing the rats under food restriction, experimental sessions began, and animals were maintained under their respective food regimen for the duration of the experiment. Water was provided ad libitum except during experimental sessions. Rats were handled daily in order to acclimatize them and minimize handling stress during the experiments. Animal care was in strict accordance with institutional and international standards of care (Animals [Scientific Procedures] Act 1986; and associated guidelines; the European Communities Council Directive [86/609/EEC, November 24, 1986] and the French Directives concerning the use of laboratory animals [décret 87–848, October 19, 1987]). All the experiments were conducted in testing rooms equipped with white noise generators.

Food

For the behavioral tests, animals were presented with two kinds of food, varying in palatability. To identify two foods that differ significantly in palatability, we first conducted a preliminary food selection experiment where a choice was given between three different types of food: laboratory chow (Scientific Animal Food & Engineering, France; containing 16.5% protein, 59% carbohydrate and 3% fat; with a caloric value of 2.9 kcal/g) and two commercial cereals: Special K (Kellogg’s, France, with 16% protein, 74% carbohydrate, and 1% fat; 3.7 kcal/g) and a chocolate-flavored cereal (Choc and Crisp, Brituggen, Germany; containing 15.2% protein, 80.8% carbohydrate, and 3.9% fat; 3.95 kcal/g as caloric value). Animals were habituated to the different foods 3 days before the development of any test to avoid food neophobia.

Experiment 1: Food Self-Selection Test

Food-sated (n = 8) and food-restricted (n = 8) rats (previously habituated to the testing environment for several days) were individually placed in a circular, black open field, measuring 45.5 cm height and 54 cm diameter, and presented with a glass bowl containing a fixed amount (15 g) of food (normal chow on the 1st day, Special K on the 2nd, and chocolate cereal on the 3rd), to habituate the animals to the testing procedure. Latency to begin eating and total food intake, taking into account any spillage, were measured over a 20-min session. On the 4th day, animals were presented with three bowls, each containing one kind of food, and were allowed 20 min to freely select their preferred food. This self-selection analysis allowed us to determine the palatability of the three food types. Normal chow and chocolate cereal differed the most in palatability and were, therefore, used in the subsequent experiments described below.

Experiment 2: Motivational Component of Feeding Behavior

The purpose of this experiment was to investigate the role of food palatability and homeostatic state on the motivation to obtain food reinforcement, measured in the runway paradigm. The runway apparatus consisted of an acrylic straight alley (180 cm long × 14 cm wide × 30 cm high), with a start box (19 × 14 × 30 cm) attached to one end. A sliding door separated the start box from the runway. Either normal chow or chocolate-flavored cereal was delivered into a glass bowl located at the end of the runway opposite to the start box. Ad libitum (n = 8) and food-restricted (n = 8) rats received 10 runway trials per day. On a given day, each rat was placed in the start box, with the door closed. When the head of the animal was pointing to the end of the alley, the door was opened and an observer counted the time each rat made to arrive at the end. There, animals were allowed to eat just for 2–3 s, to avoid early satiation. A 60-s cut-off time was used when animals did not arrive at the end of the alley. Tests were conducted with normal chow on the first 3 days and with chocolate-flavored cereal on the next 3 days. We assumed that the amount eaten by the animals was negligible, and therefore, the complete daily ration (17 g) was given after testing sessions.

Experiment 3: Anticipatory Component of Feeding Behavior

In this experiment, we evaluated the conditioned locomotor activity that occurred prior to food presentation in food-sated and food-restricted rats that were presented with normal chow or chocolate-flavored cereal.

Thirty-two rats were used in this experiment. All tests were conducted from 10 a.m. to 12 noon. and were carried out in the dark. In all cases, recording locomotor activity was restricted to the first 30 min.

Activity was tested in 32 individual cages (35 cm long × 25 cm wide × 25 cm high), with door, floor, and ceiling constructed of wire mesh and side walls made of 10-mm transparent Plexiglas (Imetronic, France). A set of two photoelectric infrared cells was placed 14 cm apart (3 cm above the floor) so that each passage of an animal from one side of the cage to the other could be detected and recorded by a computer. Another set of two photoelectric infrared cells was placed 14 cm apart (13 cm above the floor) in order to detect rearing activity. General activity (total horizontal beam breaks for each photoelectric cell), ambulation (cross-over between the inferior beams), and rearing (breaks of beams placed high) were quantified in 5-min bins with a computer program (Imetronic, France).

Habituation. Animals were placed in the locomotor activity cages for 120 min daily, for 10 days. During the habituation phase, home cage feeding occurred at varying and unpredictable intervals (2–6 hr) after testing.

Anticipatory activity. During the anticipatory activity phase, which lasted 10 days, animals were fed in the activity cages 30 min after their
Introduction into the cage. Animals were divided into four groups: ad libitum rats fed with normal chow (n = 8), ad libitum rats fed with chocolate cereal (n = 8), food-restricted rats fed with normal chow (n = 8), and food-restricted rats fed with chocolate cereal (n = 8). The amount of food delivered was 17 g of normal chow (daily ration) and 13 g of chocolate cereal, to equilibrate caloric intake. No feeding occurred in the home cages during this phase. The rats that were fed ad libitum are henceforth referred to as food-sated rats.

Extinction. Extinction sessions were carried out over 9 days, for 120 min daily. After the first 30 min of recording, no food was presented to the animals. Instead, the animals were fed in their home cages with their daily ration (17 g).

Statistical Analysis

The results were analyzed with a mixed-design multivariate analysis of variance (MANOVA), with state (food-sated vs. food-restricted) and the kind of food received (normal chow vs. chocolate cereal) as the between-subjects variables (note that in Experiment 2, kind of food was used as a within-subject variable), and daily sessions as the within-subject variable (for rearing kinetic analysis, we also included 5-min bins from each daily session as a within-subject variable). When significant overall interactions were found, further analyses of partial interactions were carried out. Post hoc analyses were performed with a Newman–Keuls test when the initial p value was significant. All data were analyzed with Statistica software (StatSoft Inc., France). A result was considered significant if p < .05. All the results are expressed as mean ± standard error of the mean.

Results

Experiment 1

In this first experiment, we determined the palatability of three food types: normal chow and two cereals (Special K and chocolate-flavored cereal). The aim was to find two food types that differed greatly in palatability, which could then be used in subsequent experiments. When rats were presented with only one kind of food, the food-sated group began eating normal chow with a greater latency and consumed less than when given either type of cereal. As expected, food-restricted animals began eating with a short latency, with only a small difference between normal chow and cereals (data not shown). When animals were presented with all three foods simultaneously, our analysis of the latency to begin eating revealed a main effect of state, $F(1, 14) = 474.39, p < .0001$; a main effect of food, $F(2, 28) = 270, p < .0001$; and a significant State × Food interaction, $F(2, 28) = 229.03, p < .0001$. As shown in Figure 1A, food-sated rats started to eat both types of cereal significantly earlier in comparison with normal chow. Likewise, food-restricted animals began to eat chocolate cereal with a shorter latency than for normal chow. Regarding food intake, we also found main effects of food $F(2, 28) = 100.58, p < .0001$ and state, $F(1, 14) = 49.67, p < .0001$; and a State × Food interaction, $F(2, 28) = 62.38, p < .0001$. Further analysis showed that the Special K and chocolate-flavored cereals had an equivalent level of palatability for food-sated animals regarding either latency to eat or food intake. Food-restricted rats, however, preferred chocolate-flavored cereal, and for this reason chocolate cereal and normal chow were used in subsequent experiments.

Experiment 2

We next tested the motivation of the rats to reach these two chosen food types using a runway paradigm. A three-way analysis of variance (ANOVA) was computed on the data from the 3rd day of testing with the normal chow or the chocolate-flavored cereal. The analysis revealed main effects of state, $F(1, 14) = 33.65, p < .0001$, and food, $F(1, 14) = 84.06, p < .0001$, and an interaction between state and food, $F(1, 14) = 26.98, p < .0001$. Taken together, these data indicate that the animals ran faster when they

![Figure 1](image.png)
were either food-deprived or presented with the chocolate cereal. Post hoc analyses of the interaction showed that food-sated animals ran significantly slower than food-restricted animals when presented with normal chow (Figure 2A); however, when presented with chocolate-flavored cereal, they ran as fast as food-restricted animals $(p = .14)$ (Figure 2B). There was no effect of trial, indicating that by the 3rd day, running levels had reached a stable level.

**Experiment 3**

The general aim of this experiment was to study the anticipatory activity that occurred prior to food presentation in food-sated and food-restricted rats presented with normal chow or chocolate-flavored cereal.

**Habituation.** During this period, we evaluated the locomotor response of each group of rats to a novel environment (the activity cage). Activity levels of food-sated and food-restricted rats declined across 10 days of habituation (data not shown), as indicated by a significant effect of session for all parameters quantified: horizontal activity, $F(9, 252) = 79.99, p < .001$; ambulation, $F(9, 252) = 84.92, p < .001$; and rearing, $F(9, 243) = 72.63, p < .001$. There was no interaction between group and session for any of the parameters considered (data not shown), indicating that locomotor activity decreased in both groups in a similar way. There was a significant main effect of group for horizontal activity, $F(1, 28) = 15.66, p < .001$; ambulation, $F(1, 28) = 11.12, p < .005$; and rearing $F(1, 27) = 23.89, p = .001$, because of the fact that locomotor responses of food-restricted rats were initially higher than those of food-sated rats. However, both groups reached a stable level of activity before beginning the next part of the experiment (data not shown).

**Anticipatory activity.** After the initial period of habituation described above, we began giving food to the rats after 30 min in the activity cage. We found that, over 10 conditioning sessions (10 days), all three parameters of locomotor activity increased in the food-restricted groups. Of these parameters we found the strongest change in rearing; therefore, only rearing responses are presented below.

The rearing scores for each group of rats over 10 days are depicted in Figure 3A. When analyzing the data from the 30-min recording, we found main effects of session, $F(9, 252) = 21.85, p < .001$, and state, $F(1, 28) = 30.08, p < .001$, but no main effect of food, $F(1, 28) = 2.43, p = .130, ns$, and no interaction between food and state, $F(1, 28) = 1.72, p = .20, ns$. This last result shows that food-sated and food-deprived rats behave similarly when presented with either kind of food. As we found a significant Food × State × Session interaction, $F(9, 252) = 2.06, p < .05$, we analyzed partial interactions and found that in the food-sated group there was no effect of food, $F(1, 14) = 0.16, p = .698, ns$, or of session, $F(9, 126) = 1.79, p = .076, ns$, and no Food × Session interaction, $F(9, 126) = 1.61, p = .119, ns$, indicating a lack of anticipatory activity. In the food-restricted groups, there was a significant effect of session, $F(9, 126) = 9.59, p < .001$, but no main effect of food, $F(1, 14) = 2.28, p = .133, ns$, and no interaction between food and session, $F(9, 126) = 1.64, p = .111, ns$. Taken together the results of this experiment show that conditioned anticipatory activity is dependent on the homeostatic state of the animal and not on the kind of food presented because only food-restricted rats showed anticipatory activity, and this activity occurred regardless of the palatability of the food presented.

Figures 3B and 3C show the kinetics of rearing activity during the 30-min recording period for the first session (Day 1) and the

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**Figure 2.** Motivation measured with the runway paradigm. Mean running time to reach the goal box in a straight alley for food-sated and food-restricted rats. (A) Normal chow in the goal box. (B) Chocolate-flavored cereal in the goal box. Significant differences from food-restricted group are represented by asterisks, *$p < .05$, **$p < .01$, ***$p < .001$. **
Figure 3. Anticipatory component of feeding behavior. (A) Rearing activity conditioned to food presentation. Each point represents the mean number of photocell beam breaks during the 30 min immediately before food was given. On Day 0, it is possible to see the values corresponding to the last habituation session for each group. Food-restricted animals developed a conditioned augmentation in rearing activity, regardless of the kind of food, whereas food-sated animals did not. (B) Kinetic analysis of the 30-min period preceding food presentation in the first conditioning session (Day 1). (C) Kinetic analysis of the 30-min period before giving food in the last conditioning session (Day 10). Note that in the food-restricted group, rearing activity increased during the last 15 min of the recording session just prior to food presentation, regardless of food type. FS NC: food-sated rats fed with normal chow; FS CC: food-sated rats fed with chocolate cereal; FR NC: food-restricted rats fed with normal chow; and FR CC: food-restricted rats fed with chocolate cereal. Significant differences from food-sated rats presented with normal chow are represented by asterisks, *p < .05, **p < .01, and ***p < .001. Significant differences from food-restricted rats presented with chocolate-flavored cereal are represented by plus signs, +p < .05, ++p < .01, and +++p < .001.
last session (Day 10) of conditioning, respectively. In general, the rats showed an increased level of activity during the initial 5 min after being placed in the activity cage (Figures 3B and 3C). This activity declined when the rats were not trained to anticipate food (Figure 3B). After 10 days of receiving food at the end of the recording session, however, the food-restricted rats increased their rearing activity during the 15 min prior to food presentation, indicating that they anticipated food presentation. A kinetic MANOVA between the first and last days showed that there was a significant State × Session × 5-Min Bin interaction, \(F(5, 140) = 3.61, p < .005\), but no significant interaction between food, session, and 5-min bin, \(F(5, 140) = 0.48, p = .791\), ns, confirming that anticipation developed only in food-deprived rats and that the palatability of the food was not a relevant factor.

When we quantified the amount of food eaten, we found that the food-sated group ate almost the same amount as the food-deprived group when presented with the palatable food, whereas they ate very little when presented with normal chow. Values for the 10th conditioning session were as follows: food-sated group fed with normal chow \(3.63 ± 0.46\) g; food-sated group fed with chocolate cereal \(12.25 ± 0.37\) g; food-restricted group fed with normal chow \(14.75 ± 0.49\) g; food-restricted group fed with chocolate cereal \(13 ± 0\) g; State × Food interaction, \(F(1, 28) = 183.36, p < .001\). The weight of the food-restricted rats did not vary between the group fed with normal chow \(380.13 ± 3.66\) g and the group fed with chocolate cereal \(384.13 ± 7.35\) g. Similarly, weight did not vary significantly between the food-sated rats fed normal chow \(440.25 ± 4.91\) g and the food-sated rats fed chocolate cereal \(451.13 ± 10.91\) g.

**Extinction.** When food was no longer presented in the activity cages, locomotor response of animals decreased (see Figure 4), as indicated by a main effect of session, \(F(8, 216) = 22.65, p < .001\). A Session × State interaction, \(F(8, 216) = 20.98, p < .001\), indicates that the activity of food-restricted animals diminished to almost the same level as that of food-sated animals. There was no significant interaction between session, state, and food, \(F(8, 216) = 0.81, p = .59\). Post hoc analysis showed that the decrease in anticipatory activity was already significant on the second day of extinction (see Figure 4).

**Discussion**

We have studied the influence of homeostatic state (food-sated or food-restricted) and food palatability on different components of feeding behavior in the rat. Our results demonstrate that food palatability and homeostatic state differentially modulate these components.

Many studies focused on the hedonic value of food used a so-called “preferred” or “palatable” food, but the actual preference for this food was often not directly tested. In this framework, we consider of capital importance the experiment in which we measured palatability by using a choice test because it allows us to select the most preferred food. In addition to normal chow, the animals were given the choice of two different kinds of cereals (Special K and chocolate-flavored cereal) to eliminate the possibility that their choice would be simply based on novelty. Food-sated rats ate an equivalent amount of both cereals, which precludes us from dissociating between a novelty and a palatability effect. However, we found that food-restricted rats had a strong preference for chocolate cereal in comparison with normal chow and Special K. The preference shown for chocolate cereal over Special K indicates that this food is highly palatable and not

![Figure 4](image-url) Extinction of rearing activity scores during the first 30 min of anticipatory activity recording. On Day 0, it is possible to see the values corresponding to the last conditioning session for each group. Values from Days 1 to 9 represent the nine extinction sessions. FS NC: food-sated rats fed with normal chow; FS CC: food-sated rats fed with chocolate cereal; FR NC: food-restricted rats fed with normal chow; and FR CC: food-restricted rats fed with chocolate cereal. **Significantly different from the other days of extinction, \(p < .001\).
merely novel. The high level of carbohydrates and the high caloric value of both cereals compared with normal chow can account for the preference shown, as foods of high caloric value and foods containing a high level of carbohydrates are very palatable (Drewnowski, 1998; Rogers & Smit, 2000). This could be especially true for food-restricted rats, given that they chose the calorically denser food.Independently of the causes (high level of calories, carbohydrate, fat, etc.), the observed differences in behavior suggest that there are real differences in motivational properties between the tested foods. However, an independent measure of palatability, such as the evaluation of taste reactivity patterns (Berridge, 1991), may provide more reliable evidence concerning the differences observed between our two foods.

It has been shown that an enhancement of palatability level can be translated into higher level of motivation for both food-sated and food-restricted rats (Hodos, 1961). Our data support this hypothesis, as our food-sated rats behave similarly to deprived animals when presented with chocolate cereal. Thus, highly palatable food may motivate an animal to consume more food than is necessary for its metabolic needs. Food-sated rats ate large amounts of palatable food and ran almost as fast as food-restricted rats in a runway test to obtain it, in accordance with the idea of Saper et al. (2002), who postulated that “almost any mammal will eat beyond its homeostatic needs if presented with highly palatable food.” We found that even if the homeostatic state principally drives feeding behavior in food-restricted rats, the hedonic value of the food also influences motivation and consumption; food-restricted rats selected chocolate cereal over normal chow in the choice test and ran significantly faster for chocolate cereal than for normal chow in the runway test. These results are in agreement with other studies showing that the perception of palatability is augmented in food-restricted rats (Berridge, 1991; Cabanac, 1988; Cabanac & Lafrance, 1990). Nonetheless, it is worth noting that the lack of differences between sated and restricted animals presented with chocolate cereal may be underestimated by a floor effect on the running time. A possible solution to override this difficulty could be the use of a longer runway or progressive ratio paradigms. Experiments in progress in the laboratory are designed to elucidate this aspect.

In humans as well, the decision to begin a meal does not result solely from homeostatic drive but also from the emotional state of the individual, the palatability of the available food, and the necessary effort to obtain the food (Kringelbach, 2004; Toates, 1986). Research has also shown that enhancing palatability increases food intake through mechanisms that counteract those associated with satiation (Bellisle & Le Magnen, 1980; Bellisle et al., 1984; Le Magnen, 1987; Yeomans, 1996).

The opioid system has been proposed to be involved in the regulation of the perception of palatability, especially through μ receptors (Glass, Billington, & Levine, 1999; Kelley et al., 2002). In humans as well as in animals, opioid agonists increase food intake, whereas opioid antagonists decrease it, with these effects being greater when palatable food is presented (Doyle, Berridge, & Gosnell, 1993; Glass et al., 1999; Gosnell, Krahn, & Majchrzak, 1990; Yeomans & Gray, 1996; Zhang & Kelley, 2000). Studies are currently carried out in the laboratory to elucidate the implication of the opioid system in the different components of feeding behavior described here.

Dopamine has traditionally been thought to be involved in the control of food intake (Ikemoto & Panksepp, 1999; Salamone & Correa, 2002) and anticipation (Berridge & Robinson, 1998; Blackburn, Phillips, Jakubovic, & Fibiger, 1989; Peciña, Cagniard, Berridge, Aldridge, & Zhuang, 2003). This hypothesis has been constructed by means of different behavioral approaches, as runway and operant behavior models. The role of dopamine on motivation measured by means of a runway paradigm is still controversial (McFarland & Ettenberg, 1998; Peciña et al., 2003) and, for that reason, experiments focused on the role of the dopaminergic system on motivation (but also on anticipation and consumption) are being performed in the laboratory.

The anticipatory locomotor activity triggered by the periodic presentation of food has been traditionally regarded as a powerful demonstration of incentive motivation to eat (Bindra, 1968). We have used rearing activity as a measure of anticipation because among the various types of conditioned locomotor activities we saw the strongest increase in rearing activity over 10 days of conditioning sessions. These results are similar to those of Holland (1977), who also found a strong increase in rearing when conditioning rats to food presentation. Holland found that relevant conditioned stimuli (CSs; i.e., light) generate conditioned responses (CRs; i.e., rearing behavior). With time, food-reinforced visual cues (the light, or CS) come to elicit eliciting behavior (CR). This behavior is described as “CS-generated” or “orientating” behavior (Holland, 1977, 1984). In our case, the cage in which locomotor activity is measured represents the CS, whereas rearing behavior in front of the activity cage door (through which food will be delivered) can be interpreted as the CR.

Surprisingly, when anticipatory conditioned activity to food presentation was tested, the food-sated group presented with chocolate cereals did not show any augmentation in rearing scores, even if food-sated rats strongly preferred chocolate cereal to normal chow as shown by the choice test (Experiment 1), the runway test (Experiment 2), and their level of consumption of chocolate cereal in the activity cages after food delivery (Experiment 3).

A possible explanation for this result may be that conditioned anticipatory activity is not entirely controlled by the hedonic properties of the food but rather by the homeostatic state. In this case, there may be at least two different motivational processes, one implicated in response to the hedonic value of the food and the other in response to the homeostatic state of the individual. This hypothesis has already been explored, and a double dissociation of two motivational mechanisms mediating food reward (and also drug reward) in food-deprived and sated animals has been shown (Bechara, Harrington, Nader, & van der Kooy, 1992; Bechara & van der Kooy, 1992; Harrington & van der Kooy, 1992; Nader, Bechara, & van der Kooy, 1997). These authors demonstrated that bilateral ibotenic acid lesions of the tegmental pedunculopontine nucleus (a limbic system output region) blocked the conditioned preference for places associated with the presence of food (and therefore the reinforcing properties of this food) when animals were food-sated but did not do so when animals were food-deprived (Bechara & van der Kooy, 1992). In other experiments, the administration of the neuroleptic flupenthixol blocked place avoidance for places associated with hunger when animals where food-deprived but had no effect on the preference for places associated with food when animals were not deprived (Bechara et al., 1992; Harrington & van der Kooy, 1992). Regarding neuro-
transmitter systems, similar results have recently been demonstrated in humans: The opioid antagonist naloxone decreased the perceived palatability of the ingested food, whereas it did not modify the sense of hunger (Yeomans & Gray, 2002). Altogether, these results suggest the existence of at least two independent systems mediating the motivational effects of food in food-sated and food-restricted animals or in human subjects. Therefore, it is possible that the development of conditioned locomotor activity has a direct relationship with homeostatic state rather than food palatability.

Another hypothesis has been raised to explain the development of conditioned anticipatory activity, which has been summarized by Mistlberger (1994). In that review, Mistlberger postulated that the origin of this food anticipatory activity lies in a circadian food-entrainable oscillator (FEO), which is independent of the light-entrainable oscillator (in mammals, situated at the hypothalamic suprachiasmatic nucleus). This FEO would be entrained when animals are in a catabolic state, that is, maintained on a single daily meal provided at a fixed time of the day (as is the case in our experiment), whereas it is not entrained when animals are maintained ad libitum (Mistlberger, 1994; Persons, Stephan, & Bays, 1993). Presently, the identity and the location of this oscillator remains uncertain, but a recent study has pointed out that the thalamic paraventricular nucleus could play a key role in the anticipatory activity developed by food-restricted rats (Nakahara, Fukui, & Murakami, 2004). Orexinergic neurons have also been associated with an efferent pathway from the FEO in mice. These neurons might increase wakefulness and locomotor activity during food anticipatory activity episodes (Mieda et al., 2004).

Research has shown that restricted daily access to a palatable meal could entrain anticipatory wheel-running activity in free-feeding rats (Mistlberger & Rusak, 1987). Nonetheless, Mistlberger (1994) later noted that certain properties of this anticipatory activity could be dependent on the manner in which it is measured. He pointed out that the magnitude and duration of food anticipatory activity is generally greater in wheel-running apparatus, lever-pressing, and food-bin approaches and is less robust and may even be absent, for example, in classical activity cages. A similar result was found by Pecoraro, Gomez, Laugero, and Dallman (2002), who showed that daily general activity (measured by telemetric probes) seems not to be highly correlated with anticipatory wheel running. Nevertheless, these authors found that only food-restricted rats were able to develop anticipatory activity (measured also by telemetry) to the presentation of sucrose solutions.

In this context, the results observed in our study on food-restricted rats are in agreement with previous studies (Baunez, Amalric, & Robbins, 2002; Olmstead, Robbins, & Everitt, 1998; Pecoraro et al., 2002). Food-deprived animals showed a conditioned locomotor activity in the presence of both kinds of food, meaning that this activity may be more related to their deprivation state than to the palatability of the food. On the other hand, as stated earlier in this discussion, food restriction is known to increase the perception of palatability in rats. Therefore, we cannot completely rule out the possibility that the two groups of restricted animals found both aliments highly palatable and developed anticipation in consequence. A way to assess that palatability is not a contributing factor on the anticipation development might involve testing an unpalatable food (such as quinine-adulterated chow). If animals continue to anticipate this kind of food, palatability would appear not to be a relevant factor.

During the early half of the last century, it was broadly accepted that an increase in drive would always be associated with increased general activity (Dashiel, 1949). This augmentation in activity was believed to represent a response to the interoceptive state of the animal (Wada, 1922). Later, it was suggested that an increase in drive state would make the animals more responsive to their respective environment, allowing them to respond in a more vigorous or persisting way (for a review, see Bolles, 1967). On the other hand, pairing stimuli (e.g., a specific activity cage that signals the imminent delivery of food) with biologically significant events (such as food presentation) assigns these stimuli incentive properties (Bindra, 1968; Robinson & Berridge, 1993). It is important to note that a food-deprived state is known to cause the development of incentive motivation (Campbell & Sheffield, 1953) and that conditioned locomotor activity to food presentation in food-restricted rats might depend on these incentive stimuli (Bolles & Moot, 1973). It remains to be determined to what extent anticipatory activity results only from incentive learning processes and to what extent a deprivation state contributes to enhancing it.

It is worth noting that the methodology used to house and take care of the animals (especially regarding feeding) was selected to minimize the probability of food entrainment development. However, in Experiment 3 there may have been some confounding of homeostatic and circadian factors controlling the behavioral output. Food-sated rats might eat in response to circadian factors, respecting the day–night cycle. In contrast, food-restricted rats might eat independently of the cycle and instead follow homeostatic pressure, which is aperiodic, and/or eat in response to scheduled meals.

The two proposed theories to explain the origin of anticipatory activity (on the basis of food entrainment or incentive/associative learning) account for some aspects but not for the entire phenomenon (for a review, see Mistlberger, 1994). Nonetheless, an appropriate experiment to dissociate one from the other seems difficult to perform given the required protocol to elicit anticipation. As stated, it is possible that food-sated rats are more sensitive to the circadian factors controlling feeding and that food-restricted rats are principally driven by homeostatic pressure to eat. If the location of the FEO is found, it will be possible to carry out neurotoxic or electrolytic lesions on this area to test whether anticipation is completely abolished (which would indicate complete circadian control of the phenomenon).

In the present study, the disappearance of anticipatory activity by the 2nd day of extinction (when the food delivery was switched back to the home cages) seems to indicate that anticipatory activity is a very labile process. This could be due to a rapid extinction process related to the lack of conditioned stimulus–unconditioned stimulus contiguity (associative learning theory). A strong FEO damping also has been proposed as an explanation of this process (circadian theory; Mistlberger, 1994).

Our results indicate that there are some interactions between the incentive properties of food, the physiological state of the organisms, and the different types of behavior that these factors can evoke. Sated rats are highly responsive to the incentive drive generated by palatable foods, which elicit consumption and running behavior. On the other hand, food restriction will further
enhance consumption and running behavior but at the same time seems to support anticipatory responses as well.

Understanding the external (i.e., palatability) and internal (i.e., homeostatic state) factors controlling feeding behavior is important given that their deregulation may be associated with several eating disorders. In the majority of cases of bulimia nervosa, which is characterized by binge eating, the food consumed while binging is highly palatable, containing a substantial quantity of fat or carbohydrates (Hetherington, Altemus, Nelson, Bernay, & Gold, 1994; van der Ster Wallin, Norring, & Homgren, 1994). Some researchers have pointed out that consumption of sweets and other palatable foods may relieve the dysphoric effects resulting from stress and depression (Dallman et al., 2003; Rogers & Smit, 2000). Chocolate overeaters have been shown to be more depressed and to score higher on different eating disorder questionnaires (Hetherington & Macdiarmid, 1995). Recently, the contribution of palatable food to the development of obesity has been highlighted (Yeomans, Blundell, & Leshem, 2004).

With regard to homeostatic state, significant weight loss can be accompanied by physiological symptoms (cold intolerance, hair loss, etc.) and psychological symptoms (depression, irritability, etc.) that are similar to those found in patients suffering anorexia nervosa (Canetti, Bachar, & Berry, 2002).

As was pointed out in a recent review (Kringlebahl, 2004), food intake and hedonic evaluation of food have been sidelined in the past by the cognitive neurosciences. It will be important to reevaluate the roles of palatability and homeostatic state on feeding behavior, as understanding their underlying mechanisms may help control pathological feeding.

References


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