

Differential Regulation of the Consummatory, Motivational and Anticipatory Aspects of Feeding Behavior by Dopaminergic and Opioidergic Drugs

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Various aspects of feeding behavior (eg consumption, motivation and anticipation) are regulated by homeostatic and hedonic systems, and are modulated by dopaminergic and opioid brain systems. Here, we have studied the modulation of these aspects of feeding behavior by opioid and dopaminergic neurotransmission while taking into account food palatability and homeostatic state. Foods that varied in palatability were presented to either food sated or food restricted rats following injections of different doses of naloxone, an opioid receptor antagonist, or flupenthixol, a dopaminergic receptor antagonist, in behavioral paradigms that measured different aspects of feeding. Naloxone decreased food intake in a dose-dependent manner in sated rats given access to palatable food, without modifying food intake in food restricted rats. Flupenthixol did not have any effect on food intake. With regard to motivation, which was tested in a straight alley, naloxone increased the latency to reach the food only in sated rats presented with palatable food. Flupenthixol did not modify the latency of any group. Conditioned locomotor activity to repeated food presentation, a measure of anticipation, is expressed only in food restricted rats. Naloxone did not modify anticipatory activity, whereas flupenthixol decreased it only in food restricted rats presented with palatable food. These results reinforce the idea that the opioid system regulates feeding through the modulation of the perceived palatability of food. The dopaminergic system seems to be more important for the regulation of anticipatory activity related to motivationally relevant stimuli.

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INTRODUCTION

Eating-related disorders such as obesity, bulimia nervosa, and anorexia nervosa have become a problem in Western societies that urgently needs treatment and prevention policies (Davis and Claridge, 1998; Hill *et al*, 2003; Rolls, 2003). In order to regulate food intake, the central nervous system (CNS) integrates both information concerning the homeostatic state of an organism as well as environmental factors, such as circadian and seasonal rhythms, the availability of the food, and the level of stressful factors. The understanding of the central regulation of feeding

behavior is necessary to develop rational policies for the treatment and prevention of eating disorders.

Several neurotransmitter systems have been implicated in the regulation of feeding behavior. Among them, the opioid and the dopaminergic systems have received most attention. The endogenous opioid system is implicated in the control of feeding behavior in animals, as well as in humans (Glass *et al*, 1999a; Yeomans and Gray, 2002). Opioid antagonists decrease food intake whereas agonists increase it (Glass *et al*, 1999a; Söderpalm and Berridge, 2000). During the last few years, it has been proposed that the opioid system could modulate the perception of food's hedonic properties (palatability) and, thereby, food consumption (Glass *et al*, 1999a; Kelley *et al*, 2002; Weingarten and Martin, 1989). Opioid antagonists selectively reduce the consumption of a palatable food, while opioid agonists increase its consumption (Glass *et al*, 1999a; Gosnell *et al*, 1990). For example, morphine, an opioid agonist, increases the hedonic reactions elicited by sucrose in rats (Doyle *et al*, 1993). In line with these findings, consumption of palatable food modifies endogenous opioid levels in different areas of the brain (Dum *et al*, 1983; Kelley *et al*, 2003). Also, the motivation to obtain food reinforcement is decreased when enkephalin

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and β -endorphin are absent in the CNS (Hayward *et al*, 2002).

Throughout the years, dopamine has been implicated in many different behaviors. It has been proposed to mediate the sensory pleasure of reward (Wise, 1982) and incentive learning (Beninger, 1983; Di Chiara, 1998, 1999, 2002). Other authors have suggested a role in the attribution of incentive salience to different stimuli (Berridge and Robinson, 1998; Robinson and Berridge, 1993) or in the invigoration of behaviors (Ikemoto and Panksepp, 1999; Salamone and Correa, 2002). Dopamine may also play a role in the prediction or anticipation of rewarded events (Blackburn *et al*, 1987, 1989; Schultz, 2002; Schultz *et al*, 1997; Weingarten and Martin, 1989), conditioned locomotion (Jones and Robbins, 1992), as well as response selection taking into account costs and benefits (Cousins and Salamone, 1994; Salamone and Correa, 2002).

We have recently characterized the influence of food palatability and homeostatic state on three aspects of feeding in rats: consumption, motivation and anticipation (Barbano and Cador, 2005). In the present study, we tested the involvement of opioids and dopamine in these three aspects of ingestive behavior. Inasmuch as feeding is very complex, different behavioral tests were applied to rats to dissociate partially the mentioned aspects from each other. This experimental approach allowed us to show a clear dissociation between them with regard to the opioidergic and dopaminergic action. As food palatability and homeostatic state are also taken into account, our work provides a comprehensive framework on the different components of feeding behavior.

MATERIALS AND METHODS

Animals

Male Wistar rats ($n = 271$, Iffa-Credo, France) were used in this study. Groups of four rats (weighting 225–250 g upon arrival at the laboratory) were housed in clear plastic cages in an animal vivarium maintained on a reversed 12-h light–dark cycle (lights off at 09:00 hours) at a constant temperature of 23°C. Following 1 week of *ad libitum* access to standard chow, half of the animals were placed on a restricted food regimen designed to reduce their body weight to 85% of their free-feeding values. They were maintained at this reduced weight for the duration of the experiments. 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 (UK Animals (Scientific Procedures) Act, 1986; and associated guidelines; the European Communities Council Directive (86/609/EEC, 24 November 1986) and the French Directives concerning the use of laboratory animals (décret 87-848, 19 October 1987)). All the experiments were conducted in dimly lit testing rooms equipped with white noise generators.

Food and Drugs

For the behavioral tests, animals were presented with two kinds of food that differed in palatability (Barbano and

Cador, 2005). The less palatable food was normal laboratory chow (Scientific Animal Food & Engineering, France), which contains 16.5% protein, 59% carbohydrate, and 3% fat, and has a caloric value of 2.9 kcal/g. The more palatable food was chocolate-flavored cereal (Choc and Crisp[®], Brüggem, Germany), which contains 15.2% protein, 80.8% carbohydrate, and 3.9% fat, and has a caloric value of 3.95 kcal/g. Animals were habituated to the chocolate cereal 3 days before the start of any test to avoid food neophobia. They were distributed in four independent groups: sated rats presented with normal chow, sated rats presented with chocolate cereal, food restricted rats presented with normal chow, and food restricted rats presented with chocolate cereal. In experiments 1 and 2, all the groups were fed with normal chow and were presented with chocolate cereal only during testing sessions. In experiment 3, during the conditioning phase, food restricted rats presented with palatable food (17 g/day) were maintained on this diet. Food sated rats experiencing palatable food were presented with chocolate cereal only during the behavioral tests.

Naloxone hydrochloride (Sigma Chemical Co., St Louis, MO, USA) was prepared in physiological saline and injected subcutaneously at the nape of a rat's neck 5 min before a behavioral test. The doses tested were 0, 15, 120, 500, and 1000 μ g/kg.

Flupenthixol dichlorhydrate (H. Lundbeck A/S, Denmark) was also prepared in a saline solution. Based on previous studies (Agmo and Soria, 1999; Salamone and Correa, 2002), flupenthixol was administered intraperitoneally 1 h before testing. The doses administered were 0, 25, 50, 100, and 200 μ g/kg.

A Latin square design model was used, in which each animal randomly experienced all doses of either naloxone or flupenthixol and, therefore, served as its own control. At least 2 or 3 days separated the administration of each antagonist.

Experiment 1: Consumption Test

We tested the extent to which the blockade of opioid or dopaminergic receptors modifies consumption of foods that vary in palatability while taking into account the homeostatic state of the animals.

A total of 95 rats were used, 63 for the naloxone study and 32 for the flupenthixol study. The animals were habituated to the testing environment (locomotor activity cages, see description below) for several days before drug administration to ensure that there was no effect of environmental novelty on feeding behavior. On testing days, rats were placed in the cages 15 min before drug injection. At 5 min (naloxone) or 1 h (flupenthixol) postinjection, 15 g of food were presented and rats were allowed to eat for 20 min. The total amount eaten, taking into account any spillage, was measured.

Experiment 2: Motivational Component of Feeding Behavior

The purpose of this experiment was to investigate the role of dopamine and opioid neurotransmission on the motivation to obtain food reinforcement in the runway paradigm,

taking into account the palatability of the food and homeostatic state.

The runway apparatus consisted of an acrylic alley (180 cm long \times 14 cm wide \times 30 cm high) with a start box (19 \times 14 \times 30 cm) attached to one end. A sliding door separated the start box from the runway. Either normal chow or chocolate-flavored cereal was presented in a glass bowl at the far end of the runway. In all, 32 rats were used for each antagonist study. Food sated and food restricted rats received 10 runway trials per day. On a given day, each rat was placed into the start box, with the door closed. When the head of the animal was pointing to the end of the alley, the door was open and an observer counted the time each rat took to reach the end. Animals were allowed to eat for only 2–3 s, to avoid early satiation. A 60 s cutoff time was used when animals did not arrive at the end of the alley. This training procedure was repeated for 4 days and when the running behavior was stable drug test sessions began.

Experiment 3: Anticipatory Component of Feeding Behavior

This experiment examined the effects of dopaminergic (64 rats) and opioid (32 rats) antagonists on locomotor activity conditioned to food presentation in sated- and food restricted animals, presented with normal chow or chocolate-flavored cereal.

All tests were conducted from 10:00 to 12:00 hours and were carried out in the dark. In all cases, rats were presented with food 30 min after being placed in the activity cages. We showed previously that anticipatory activity occurred during the last 15 min prior to food presentation (Barbano and Cador, 2005) and, therefore, the effects of dopaminergic and opioid antagonists were evaluated within this interval.

Activity was tested in 32 individual cages (35 \times 25 \times 25 cm). The door, floor, and ceiling of each cage are made of wire mesh and the side walls are made of 10 mm thick transparent Plexiglas (Imetronic, France). A set of two photoelectric infrared cells are 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. Ambulation (crossover between the inferior beams) and rearing activity (breaks of beams placed at the top of the cage) were quantified in 5-min bins with a computer program (Imetronic, France).

Before any test, animals were habituated to the locomotor activity cages for 2 h daily, for several consecutive days. During this phase of the experiment, feeding occurred at unpredictable intervals (2–6 h) in the home cage, following the 2 h period of habituation. Once the experiment had begun (conditioning phase), rats were fed 30 min after being placed in the activity cages. The rats were given their daily ration of food (ie either 17 g of normal chow or 13 g of chocolate cereal per day with less chocolate cereal given to equilibrate caloric intake) and had 90 min to eat. No feeding occurred in the home cages. After 10 days of this protocol, when the conditioned activity level had stabilized, the effects of naloxone or flupenthixol were assessed.

Experiment 4: Effect of Opioid and Dopaminergic Antagonists on General Motor Activity

To test for any motor impairment naloxone or flupenthixol might have on general motor activity, we tested their action on unconditioned locomotor activity. In all, 48 naive rats (ie animals that had never been conditioned in the testing environment) were given naloxone, and 32 naive rats were given flupenthixol. Locomotor testing began 5 min after naloxone injection and 1 h after flupenthixol injection. For the naloxone study, ambulation was recorded for 20 min; and for flupenthixol study, rearing activity was recorded for 30 min. As we observed an effect of flupenthixol during the last 15 min of this 30 min period, only this portion of records was analyzed to detect motor impairment due to the neuroleptic.

Statistical Analysis

Straight alley test data were analyzed with nonparametric methods because the distributions of variables were not normal (due to the cutoff applied), while the other behavioral tests were analyzed with parametric methods. In runway tests, we used Kruskal–Wallis ANOVA to compare the four different groups and Friedman ANOVA to detect any intertrial difference for a given group. To perform between-group comparisons, the Mann–Whitney *U*-test was performed, while the Wilcoxon matched pairs test was used when performing within-group comparisons.

For the other behavioral tests, data were analyzed using a mixed-design multifactorial analysis of variance (MANOVA), with state (sated vs food restricted) and kind of food (normal chow vs chocolate cereal) as between-subject factors, and drug doses as within-subject factor. For significant overall interactions, further analyses of partial interactions were carried out. *Post hoc* analyses were performed with 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 $p < 0.05$. All the results are expressed as mean \pm SEM.

RESULTS

Experiment 1

We first measured the effects of naloxone, an opiate antagonist, and flupenthixol, a dopamine receptor antagonist, on the consummatory aspects of feeding behavior by measuring food intake. Under control conditions (saline injection, Figure 1a and b), sated rats presented with palatable food ate as much as food restricted rats presented with palatable food, while restricted rats presented with normal chow had a lower intake level than either of these two groups. As expected, sated rats presented normal chow ate the less.

The effect of naloxone on consummatory responses is shown in Figure 1a. MANOVA showed a main effect of food ($F_{1,59} = 144.18$, $p < 0.001$), state ($F_{1,59} = 127.67$, $p < 0.001$), and dose ($F_{4,236} = 30.62$, $p < 0.001$). All the interactions were significant (ie food \times state \times dose: $F_{4,236} = 3.93$,

$p < 0.005$). Further analyses of the sated groups showed that naloxone decreased food intake the most in the cereal-fed group (food \times dose interaction: $F_{4,120} = 7.79$, $p < 0.001$). Significant reduction of food intake occurred from the dose $120 \mu\text{g/kg}$ onwards in the cereal-fed group and from the dose $500 \mu\text{g/kg}$ onwards in chow-fed rats. For food restricted rats, there was no state \times dose interaction, but a main effect of dose ($F_{4,116} = 4.55$, $p < 0.005$). Newman-Keuls test showed that the only dose that significantly differs from control groups was $1000 \mu\text{g/kg}$.

Flupenthixol did not have any effect on consummatory responses as can be seen in Figure 1b. We found a main effect of state ($F_{1,27} = 45.64$, $p < 0.001$), of food ($F_{1,27} = 60.71$, $p < 0.001$), but no main effect of dose ($F_{4,108} = 0.28$, $p = 0.89$, NS) and no interaction with dose (ie state \times food \times dose: $F_{4,108} = 0.31$, $p = 0.87$).

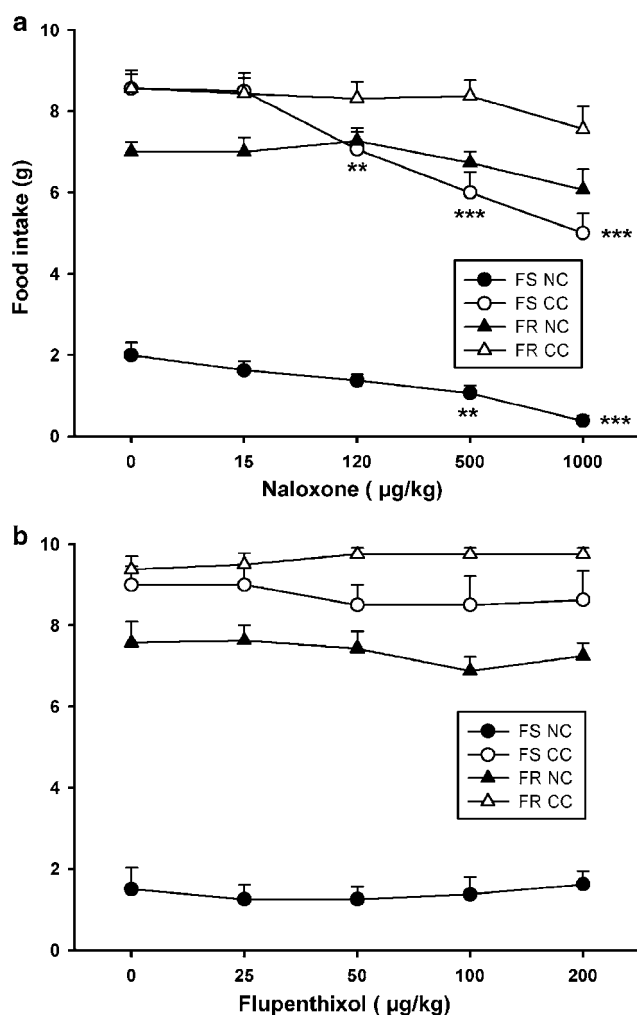


Figure 1 Effect of naloxone (a) and flupenthixol (b) administration on the consummatory component of feeding behavior. The food sated rats that were fed chocolate cereal ate progressively less with increasing doses of naloxone. The asterisks indicate a significant difference from the vehicle solution ($0 \mu\text{g/kg}$). 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 flavored cereal. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Experiment 2

We determined the effects of naloxone and flupenthixol on the motivation to reach food by measuring the time it took to run the length of a straight alley. When saline was administered to rats (Figures 2a and b), the food sated group presented with normal chow showed no interest in reaching the goal box, while the sated group presented with chocolate cereal and both food restricted groups took equivalent amounts of time to run the length of the alley.

Naloxone decreased motivation only in the sated, cereal-fed group (Figure 2a). Mann-Whitney U -test revealed that food sated and food restricted rats did not differ when presented with chocolate cereal under control conditions (saline injection). Kruskal-Wallis analysis of variance (ANOVA) showed that there were significant differences between the four experimental groups with regard to each dose of naloxone. When Friedman ANOVA was performed to assess the effect of drug administration, there was no difference for sated, chow-fed animals ($\chi^2_4 = 8.08$, $p = 0.09$, NS), food restricted chow-fed animals ($\chi^2_4 = 2.84$, $p = 0.58$,

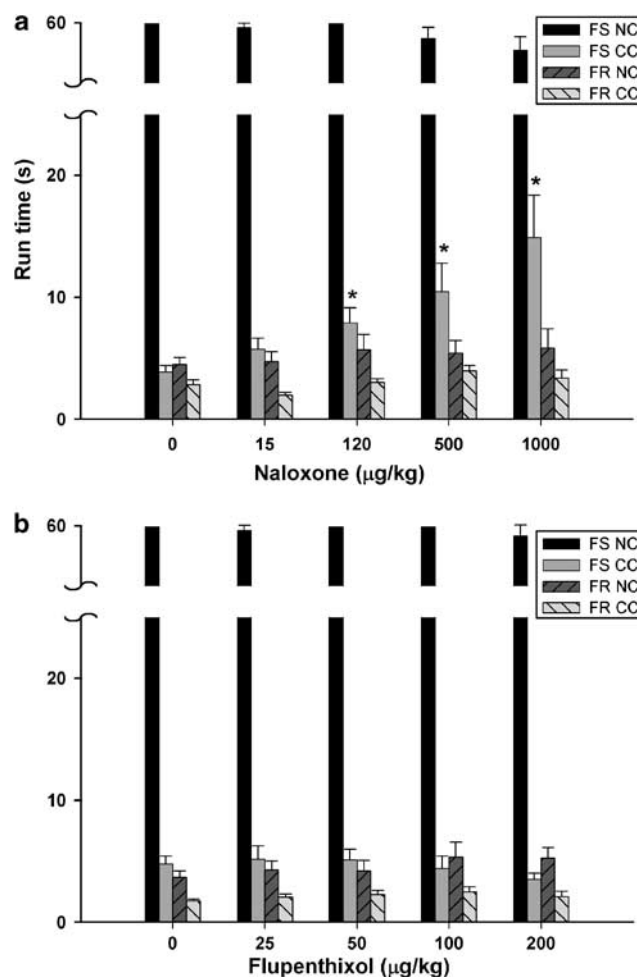


Figure 2 Effect of naloxone (a) and flupenthixol (b) administration on the motivational component of feeding behavior, measured by means of the straight alley paradigm. Naloxone increased run time only in FS CC group, indicating a decrease in motivation to reach the food reward. Legends as in Figure 1. * Significantly different with respect to the dose $0 \mu\text{g/kg}$ of naloxone ($p < 0.05$).

NS), or food restricted cereal-fed animals ($\chi^2_4 = 6.38$, $p = 0.17$, NS), but there was a significant effect of naloxone on running time for food sated cereal-fed group ($\chi^2_4 = 16.123$, $p < 0.005$).

As in the consumption test above, flupenthixol had no significant effect in this behavioral paradigm (Figure 2b). Kruskal-Wallis ANOVA showed that there were significant differences between the four experimental groups with regard to their running performance, but Friedman ANOVA did not reveal any effect of drug administration (sated, chow-fed group: $\chi^2_4 = 4.00$, $p = 0.41$, NS; sated, cereal fed group: $\chi^2_4 = 2.79$, $p = 0.59$, NS; food restricted, chow-fed animals: $\chi^2_4 = 1.90$, $p = 0.75$, NS; or food restricted, cereal-fed animals: $\chi^2_4 = 1.93$, $p = 0.75$, NS). This means that the different groups had different running performances following saline injection and that flupenthixol did not modify these differences.

We plotted the performances across trials and doses to see if there were any intertrial differences. The effect of naloxone increased with trial number in the food sated rats presented with chocolate cereal; the mean run time increased significantly after the third trial and continued to augment thereafter (Figure 3a). With regard to the other groups, no modification was seen after naloxone administration. There were no significant intertrial differences with flupenthixol at any dose. In Figure 3b, the run time after the injection of a high dose of flupenthixol was equivalent to the run time after saline administration.

Experiment 3

In this experiment, we measured the effects of naloxone and flupenthixol on the anticipatory component of feeding behavior. In a previous study, we found that, after being placed in cages designed to measure locomotor activity, rats show an increase in activity in the 15 min interval just prior to food presentation (Barbano and Cador, 2005). As the strongest anticipatory response was seen for rearing behavior, only this response will be presented here. However, equivalent results were also obtained with ambulation (data not shown).

Under control conditions (saline injection), food restricted rats developed an anticipatory activity conditioned to food presentation, regardless of the kind of food presented, as observed previously (Barbano and Cador, 2005). In contrast, food sated rats presented with palatable food did not develop any anticipatory activity, despite the fact that they ate and ran as much as the food restricted groups in the consumption and motivation experiments, respectively.

As can be seen in Figure 4a, naloxone did not decrease anticipatory activity conditioned to food presentation. MANOVA showed only a main effect of state ($F_{1,28} = 94.98$, $p < 0.001$) and no main effect of dose ($F_{4,112} = 2.07$, $p = 0.09$, NS). There was also no main effect of food ($F_{1,28} = 0.91$, $p = 0.35$), meaning that the animals behave in a similar way regardless of the type of food

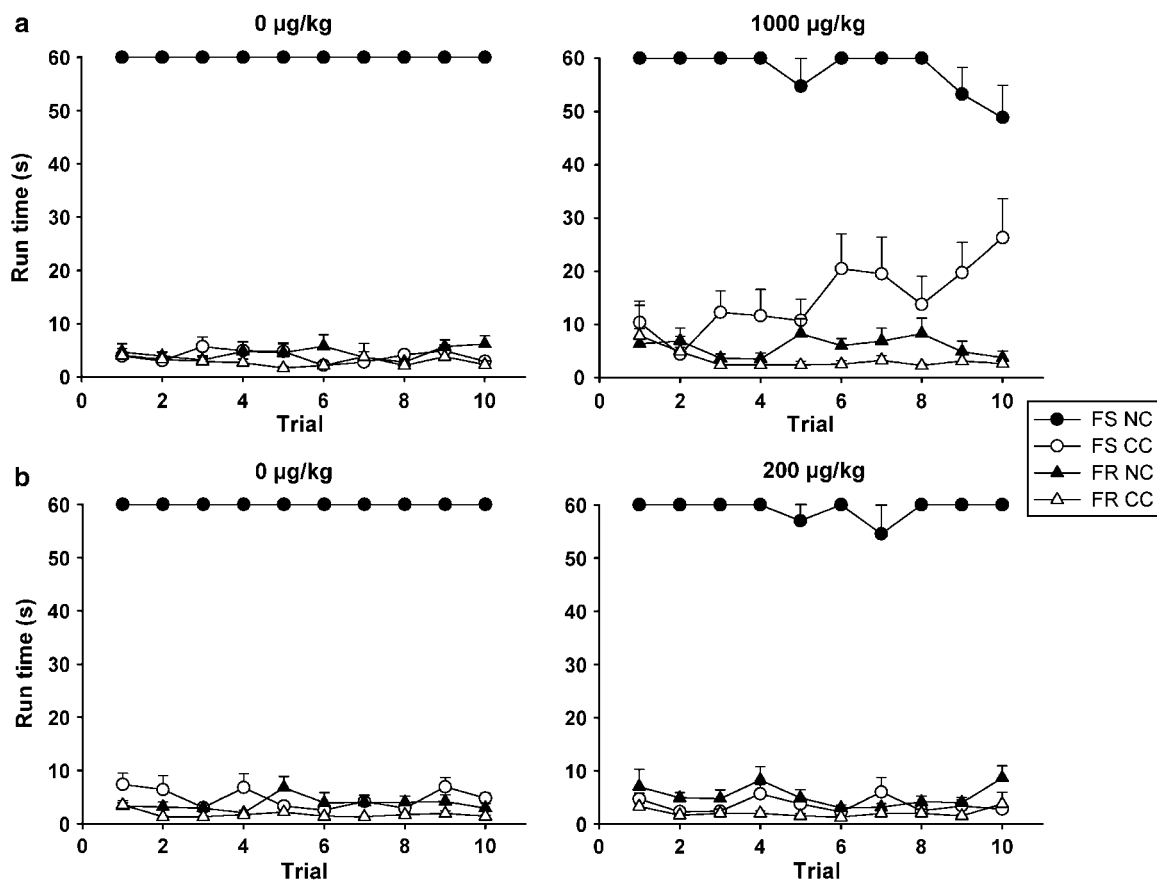


Figure 3 Intertrial variation in the effect of naloxone (a) and flupenthixol (b) on motivation for food reinforcement. Naloxone increased run time with trial number in the FS CC group. Each point represents the mean of eight animals for a given trial. Legends as in Figure 1.

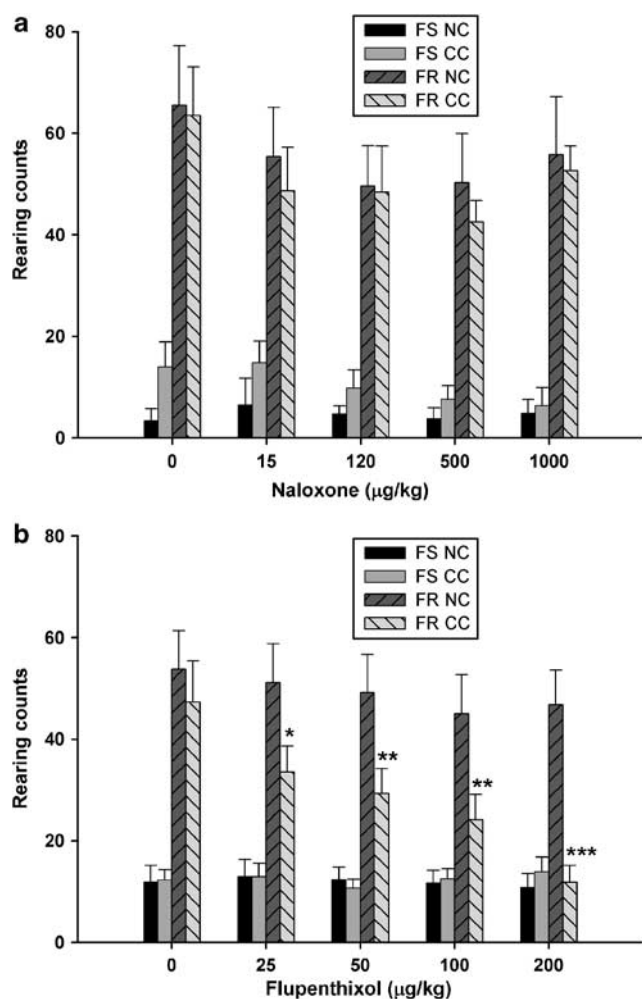


Figure 4 Effect of naloxone (a) and flupenthixol (b) administration on the anticipatory component of feeding behavior. Flupenthixol decreased rearing in the FR CC group. Legends as in Figure 1. * ** *** Significantly different from dose 0 µg/kg of flupenthixol ($p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively).

presented. None of the resultant interactions reached significance (ie dose \times state \times food: $F_{4,112} = 0.16$, $p = 0.96$).

Contrary to its lack of effect on consummatory and motivational responses, flupenthixol decreased anticipatory activity in food restricted cereal-fed rats (Figure 4b). However, it had no effect on food sated rats or food restricted rats that were fed with normal chow. After performing an overall MANOVA, we found a dose \times state \times food interaction ($F_{4,228} = 2.64$, $p < 0.05$), which allows us to perform partial analyses. In addition to this interaction, we found a main effect of state ($F_{1,57} = 39.76$, $p < 0.001$) because the level of activity in food restricted group remained elevated in comparison to the food sated group and a main effect of food ($F_{1,57} = 5.74$, $p < 0.05$), indicating that the activity scores were higher for normal chow than for chocolate cereal. We also found a main effect of dose ($F_{4,228} = 5.81$, $p < 0.001$), which means that flupenthixol decreased activity in a dose-response way. Analyses of partial interactions showed that food sated rats did not modify their activity scores when injected with the flupenthixol (effect of dose: $F_{4,120} = 0.10$, $p = 0.98$, NS),

while food restricted rats did (effect of dose: $F_{4,120} = 7.84$, $p < 0.001$; dose \times food interaction: $F_{4,120} = 2.86$, $p < 0.05$). Newman-Keuls test revealed that the activity scores of food restricted rats fed with chocolate cereal diminished in a dose-dependent manner (this decrease was significant starting at a dosage of 25 µg/kg), while the activity scores of food restricted rats fed with normal chow did not change.

Experiment 4

To examine the possibility that the results obtained with naloxone and flupenthixol are simply due to motor effects, we tested independent groups of rats, in the same activity cage used in experiment 3. These rats were not given food after being placed in either cage. Thus, a change in their locomotor activity after drug administration would not be related to anticipation but rather inherent and unconditioned.

Since naloxone increased run time within a straight alley for one group of rats (food sated, cereal fed), here we tested the general effects of naloxone on ambulation. Likewise, since flupenthixol decreased rearing in one group of rats (food restricted, cereal-fed), here we tested the general effects of flupenthixol on rearing.

To test naloxone, we looked at ambulation (crossover counts) within the same temporal window (20 min) during which the drug effectively decreased consumption (both food sated groups) and run time (food sated, cereal-fed group). When the effect of naloxone was tested (Figure 5a), we did not find a main effect of state ($F_{1,14} = 0.03$, $p = 0.86$) or of dose ($F_{4,56} = 2.30$, $p = 0.07$). The dose \times state interaction was also not significant ($F_{4,56} = 0.86$, $p = 0.49$). These results indicate that naloxone had no effect on the unconditioned motor activity during the interval in which motivation and consumption tests were performed.

To test flupenthixol, as in our other experiments, flupenthixol was administered intraperitoneally 1 h before testing. The rats were then placed in the activity cage and rearing activity was measured for a 30 min period and data from last 15 min were analyzed (this 15 min period is equivalent to the interval in which flupenthixol decreased anticipatory activity for food restricted, cereal-fed group). Unlike experiment 3, in this experiment the rats were not given food in the activity cage at the end of the 30 min period, and they did not develop an anticipatory response (see Barbano and Cador, 2005). Flupenthixol did not modify rearing scores during the 15 min interval (Figure 5b). We did not find a main effect of state ($F_{1,25} = 2.69$, $p = 0.11$) or dose ($F_{4,100} = 1.29$, $p = 0.28$), nor a dose \times state interaction ($F_{4,100} = 0.18$, $p = 0.95$). Altogether, the results indicate that the effects observed on anticipatory activity were not due to any locomotor impairment.

DISCUSSION

In order to understand the neural substrates of feeding behavior, it is necessary to study multiple components of this complex behavior in parallel. Here, we studied the role of dopaminergic and opioid neurotransmission on different components of feeding behavior (anticipation, motivation and consumption), while taking into account food palat-

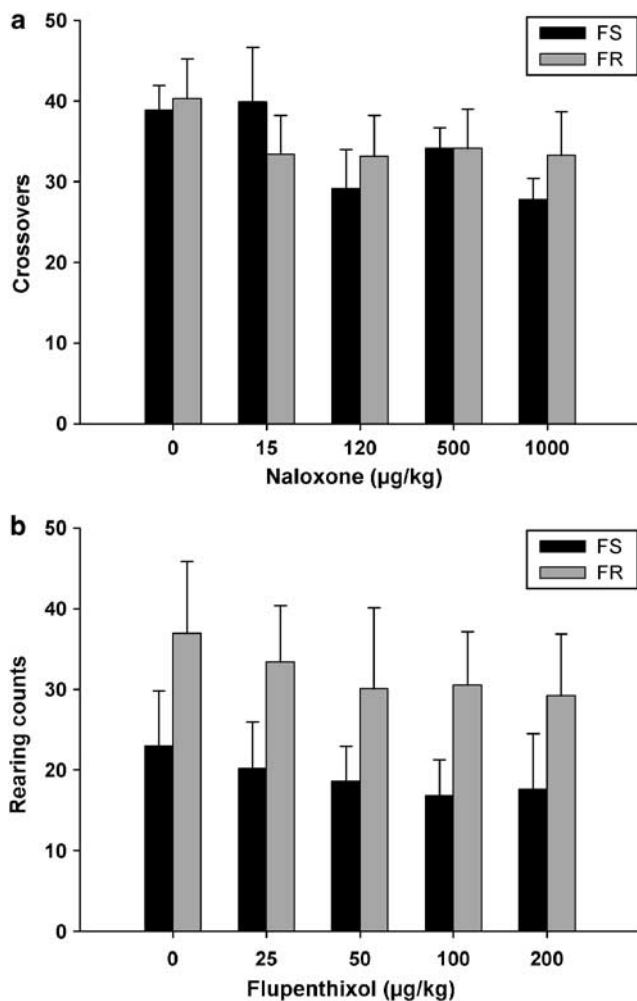


Figure 5 The effect of naloxone (a) on unconditioned ambulation and of flupenthixol (b) on unconditioned rearing activity. Naloxone administration did not modify ambulation in the 20 min period, corresponding to the time when consumption and straight alley tests were done. Flupenthixol did not have a significant effect on rearing during the 15 min interval, corresponding to the interval in which it effectively decreased anticipation. FS: food sated rats; FR: food restricted rats.

ability and homeostatic state. Our data show that these two neurotransmitter systems differentially regulate the various components of feeding behavior.

We found that the opioid antagonist naloxone decreased consumption in general in a dose-dependent manner in sated animals and was particularly effective at reducing intake of palatable food. Similar results were observed in rats given unrestricted access to sucrose or saccharin solutions or sweet chow (Cleary *et al*, 1996; Levine *et al*, 1995; Lynch, 1986). In humans, naltrexone (an opioid receptor antagonist) administration decreases the pleasantness of the food, without affecting the rated appetite prior meal initiation (Yeomans and Gray, 1997, 2002). It has been proposed that the decreased food intake after opioid receptor blockade could be related to an altered sensory perception, but previous experimental data do not support this hypothesis (Arbisi *et al*, 1999; Drewnowski *et al*, 1992).

Hutchinson *et al* (2000) showed that a high dose of naloxone (5.6 mg/kg), elicited a taste aversion to a saccharin solution, while doses ranging between 1 and 3.2 mg/kg were

ineffective. In our study, it is unlikely that taste aversion is responsible for the observed decrease in consumption since the maximum dose was 1 mg/kg. In addition, other authors have shown that naloxone induced conditioned taste aversion only at high doses, while anorectic effects of naloxone are observed with doses much lower (Hunt *et al*, 1983; Leshem, 1984; Lynch, 1986). On the whole, our data are in accordance with the hypothesis that the opioid system regulates feeding through the modulation of the perception of food palatability.

We found that food intake in food restricted animals was almost unaffected by naloxone administration. Giraudo *et al* (1993) showed that naloxone decreased consumption in rats subjected to 18 or to 48 h of acute food deprivation. The decrease in food intake was much greater for very palatable food compared with normal chow. In accordance, Levine *et al* (1995) demonstrated that rats acutely deprived reduced intake of palatable food following naloxone, in comparison to rats chronically food restricted. These authors, as well as Rudski *et al* (1994), state that the anorectic effects of naloxone are inversely correlated with the deprivation state of the animals (ie naloxone is less effective when the deprivation degree is higher or when animals are deprived chronically). Altogether, our results and the evidence in the literature suggest that feeding in response to metabolic needs is much less dependent, if at all, on the opioid system than feeding motivated by palatability. It is likely that in sated rats, opioids would mediate the hedonic value of food reinforcers, whereas in the deprived state, the opioid system would be superseded by the systems that regulate feeding in response to metabolic energy requirements.

When motivation to obtain food reinforcement was measured in a runway paradigm, naloxone administration increased the time to reach the goal box in sated rats presented with palatable food. Naloxone did not have any effect on the other experimental groups. Sated rats presented with normal chow showed so little motivation to reach the goal box that naloxone could not have a detrimental effect on their performance. The lack of effect of naloxone on food restricted rats, which consistently ran fast to reach the goal box when presented with either normal chow or chocolate cereal, is in accordance with our results presented above for consumption as well as with previous works (Kirkham and Blundell, 1986; Nencini and Graziani, 1990). In these studies, starting and running speed were not significantly modified by naloxone or naltrexone, even when a high dose of naloxone (5 mg/kg) was used.

The fact that food sated rats presented with chocolate cereal run as fast as food restricted rats confirms the results of our previous work (Barbano and Cador, 2005). To our knowledge, this is the first study in which the role of an opioid antagonist was assessed in food sated rats that were presented foods differing in palatability. The earliest works on the role of the opioid system on motivation, measured by the runway paradigm, did not generally test sated animals (Kirkham and Blundell, 1986; Nencini and Graziani, 1990). Lately, using an alternative paradigm, the progressive ratio schedule, it has been shown that food sated animals were much more sensitive to naloxone than food restricted animals (Hayward and Low, 2001; Rudski *et al*, 1994). The same results have been observed in knockout mice lacking enkephalin, β -endorphin or both opioid peptides (Hayward

et al, 2002). All these findings are consistent with the idea that the opioid system regulates the component of feeding behavior driven by the hedonic rather than the metabolic value of food. Another observation that strengthens this hypothesis is that the effect of naloxone was not apparent during the first runway trials (Figure 3a). Kirkham and Blundell (1986) have found similar results, as did Glass *et al* (1999b), in a study of obese and lean Zucker rats. It seems that rats need to experience food under naloxone to show the observed effects. If food is no longer perceived as palatable, a decrease in the motivation to obtain it would be a logical consequence.

In a previous study, we found that only food restricted animals developed anticipatory activity prior to food presentation (Barbano and Cador, 2005). Here, we show that naloxone did not affect the expression of anticipatory activity in food restricted animals presented with either normal chow or chocolate cereal. The few existing data so far are contradictory (Mistlberger, 1994; Shido *et al*, 1986). A recent work showed that mice lacking the μ -opioid receptor have a decreased level of anticipatory wheel running activity compared with wild-type or heterozygote mice (Kas *et al*, 2004). Nevertheless, the authors tested the mice only over 4 days, during the development of anticipatory wheel running and not after stabilization. These mice showed a decreased response, but not a lack of response. If the mice were tested longer, it might well be that the initial effect would become less evident. More work is needed to elucidate the role of the opioid system on food anticipatory activity given the controversial results obtained to date. In our case, acute naloxone administration had no effect on fully developed, stable anticipation, suggesting that the opioid system is not involved in the *expression* of anticipatory activity to food presentation. Nevertheless, we cannot rule out the possibility of opioid system involvement on anticipatory activity *induction*.

When naloxone effects were tested on unconditioned ambulation, no motor deficit was observed. The range of doses used was chosen based on previous studies of our laboratory, indicating that these doses were not aversive *per se* (Frenois *et al*, 2002). The lack of effect of naloxone on ambulation excludes the possibility that the observed effects in the runway or in the consumption test could result from motor impairment. Also, naloxone had no effect on rearing activity, which was a measure of anticipation. Altogether, our results indicate that the observed effects of naloxone might be specific to the hedonic perception of the food.

Dopamine has been proposed to code for the rewarding properties of different stimuli, such as food, sex, and drugs of abuse (Hernandez and Hoebel, 1988a,b; Kiyatkin, 1995; Melis and Argiolas, 1995; Wise, 1982), but recent works have contested this hypothesis. For example, the administration of selective dopamine D1 and D2 antagonists into different subregions of the nucleus accumbens decreased locomotor activity but did not impair food intake (Baldo *et al*, 2002). Treit and Berridge (1990) showed that moderate doses of the neuroleptic haloperidol do not modify the hedonic or aversive reactions to sucrose or quinine solutions in rats. Similarly, Salamone *et al* (1991) demonstrated that haloperidol administration (or dopamine depletion of the nucleus accumbens) did not change the amount eaten or the preference for either palatable food

(Bioserve pellets) or laboratory chow. A similar result was found by Cannon and Palmiter (2003, see also Cannon and Bseikri, 2004), who showed that dopamine was not necessary for mice to show a preference for rewarding saccharin or sucrose solutions. In our study, dopaminergic blockade with flupenthixol did not modify food intake in any experimental group, even in the groups fed with palatable food, showing that the consummatory and motivational aspects of food reward were intact. This result is not surprising since it has been shown that low doses of neuroleptics, which decrease the operant response to food, do not in general modify food intake, although increases are sometimes seen (Salamone and Correa, 2002; Salamone *et al*, 1991; Weingarten and Martin, 1989). Higher doses of neuroleptics or nigrostriatal dopamine depletions have been shown to decrease food intake, but these effects are rather due to an action on the movement and motor coordination required for eating (Berridge *et al*, 1989; Jicha and Salamone, 1991; White, 1986). Altogether, the present findings support the idea that dopamine is not essential to evoke consummatory behaviors.

We did not find any effect of flupenthixol on the motivation to reach food for any experimental group as assessed using a runway paradigm. As dopamine has been implicated in incentive motivation (Berridge and Robinson, 1998; Fibiger and Phillips, 1986; Mogenson and Phillips, 1976; Robinson and Berridge, 1993) and incentive learning (Di Chiara, 1998, 1999, 2002), these data may seem at first unexpected. Nevertheless, recent findings show that many aspects of food motivation are spared after neuroleptic administration. Using the same behavioral paradigm, McFarland and Ettenberg (1998) showed that the dopamine antagonist haloperidol did not modify the mean run time in food restricted rats trained to traverse a straight alley for food reinforcement. More interestingly, these authors used two discriminative olfactory cues that allowed the rats to predict the presence or absence of a reward. The neuroleptic failed to disrupt the discrimination between these two cues; rats continued to run faster when presented with the food-predictive cue. Another study using a T-maze task showed that rats given the choice of a high reinforcement density (HD) in one arm and a low reinforcement density (LD) in the other will continue to choose the HD arm after haloperidol injection or nucleus accumbens dopamine depletion (Salamone *et al*, 1994). However, when a barrier, which obliged the rats to climb, blocked the HD arm, dopamine-depleted or neuroleptic-treated rats significantly decreased the number of times they selected the HD arm. In the same line, haloperidol or nucleus accumbens dopamine depletion effects were much stronger when a highly demanding motor task was required for food obtainment (Cousins and Salamone, 1994; Salamone *et al*, 1991). All these data seem to indicate that dopamine is particularly involved in highly motor demanding tasks, which are more sensitive to motivational levels.

Ikemoto and Panksepp (1996) showed that *cis*-flupenthixol infusions into the nucleus accumbens or the ventral tegmental area decreased running speed while leaving sucrose intake unchanged, a result different from the one obtained by us. Nevertheless, this work differs from our study on several points: the type of reinforcement, the shape of the runway, the homeostatic state of the animals, the

number of experimental trials during training and testing, the doses, and the route of administration used. The J-shaped runway used in their study was larger than our straight alley and had a bend in it. These differences may increase the difficulty of the motor task and consequently make the task more susceptible to disruption by neuroleptic administration.

When anticipatory activity was assessed, only food restricted rats showed an augmentation in rearing during a 15 min period prior to food presentation that was independent of food type, as shown previously (Barbano and Cador, 2005). The level of activity for both restricted groups was equivalent in control conditions. Flupenthixol did not have any effect on the activity of rats anticipating normal chow, but significantly reduced the activity of rats anticipating a very palatable meal. In the literature, the role of dopamine in anticipatory or preparatory components of feeding behavior remains uncertain (Blackburn *et al*, 1987; McCullough and Salamone, 1992; Mistlberger, 1994; Mistlberger and Mumby, 1992; Salamone, 1988; Weingarten and Martin, 1989). No effect of dopamine depletion or neuroleptic treatment is evident when normal chow is presented to deprived animals (Jones and Robbins, 1992; Mistlberger and Mumby, 1992). On the other hand, several studies that did show the involvement of dopamine in anticipatory activity related to feeding were carried out using food other than normal chow and in most of the cases, palatable food (Blackburn *et al*, 1987, 1989; McCullough and Salamone, 1992; Salamone, 1988; Weingarten and Martin, 1989). Our data suggest, for the first time, that food palatability might be a critical parameter in the dopaminergic modulation of anticipatory activity in restricted animals.

It has been proposed that motivation comprises different aspects: directional and activational (Cofer, 1972; Duffy, 1963). Directional aspects are defined as 'the selection of a particular response that is directed toward or away from a particular stimulus', while activational aspects refer to 'quantitative features of the behavior, such as vigor, amplitude, rate or maintenance, that are demonstrated in the execution of a response' (Salamone, 1988). Directional aspects of motivation seem to be spared after dopaminergic antagonism. On the contrary, activational aspects of motivation seem to be very susceptible to dopamine disruption, at least when palatable food is presented to rats (our results; Salamone, 1988). If that is the case, why dopamine blockade did not modify anticipatory activity of rats anticipating normal chow?

A possible explanation may be found in the interpretation of dopamine's role in motivation by Ikemoto and Panksepp (1999). These authors propose that nucleus accumbens dopamine may modulate a flexible response system (a system that operates when animals are learning about incentive contingencies in their environment) (Ikemoto and Panksepp, 1999, p. 16) in the presence of *salient* stimuli. This system, in turn, would generate a state of incentive motivation and exploratory arousal toward incentive and novel stimuli. As suggested by the authors, dopaminergic disruption should blunt the ability of organisms to be aroused by salient stimuli (equivalent to the activational component of motivation blunted by dopaminergic antagonism proposed by Salamone). It can be hypothesized that

learning the conditioned-unconditioned stimuli contingency that elicits anticipatory activity is initially dopamine-dependent. After the learning period, anticipation of very salient stimuli still depends on dopamine, while anticipation of habitual, not novel stimuli does not. This hypothesis needs to be further explored but findings in the literature and our own data seem in accordance with a role of dopamine on the anticipatory activity related only to palatable, salient food.

In conclusion, an understanding of the neural substrates of feeding may require studies in which several components of this complex behavior are studied concurrently. Here, different behavioral tests were applied to rats in an attempt to dissociate the various components from each other. In addition, food palatability and homeostatic state were also taken into account. This experimental approach has allowed us to show a clear distinction between the action of opioid and dopaminergic antagonists. The involvement of the opioid system in the modulation of food's hedonic properties seems to be well established with regard to feeding behavior, specifically consumption and motivation. On the other hand, the role of dopamine still remains elusive. We believe that there is not a unique role of dopamine in the generation of a given behavior because many diverse processes are sensitive to this neurotransmitter (eg learning, motor performance, incentive attribution to neutral stimuli, evaluation of the cost-benefit of performing an action, behavioral activation, among others). The aim of future research should be to reconcile the proposed theories regarding the role of dopamine on behavior, focusing on the strong points of each one. We will then be closer to comprehending the neural control of feeding.

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