

# A Pharmacological Analysis of the Substrates Underlying Conditioned Feeding Induced by Repeated Opioid Stimulation of the Nucleus Accumbens

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It has previously been demonstrated that stimulation of opiate receptors within the nucleus accumbens results in marked hyperphagia, perhaps reflecting enhancement of taste palatability. Rats that have received multiple morphine treatments also increase feeding in response to environmental stimuli that have been associated with the morphine injections. The present investigation further examined this phenomenon. In Experiment 1, it was shown that induction of conditioned feeding was dose-dependent; significant conditioned feeding was obtained with repeated ( $n = 5$ ) intra-accumbens injections of 5 or 10  $\mu\text{g}/\mu\text{l}$  morphine but not with saline or 1  $\mu\text{g}$ . The conditioned feeding response was blocked by systemic naltrexone (5 mg/kg). In the second experiment, co-treatment with either a D-1 (SCH 23390, 0.1 mg/kg) or D-2 (haloperidol, 0.25 mg/kg) antagonist did not block the development of conditioned feeding, nor did these drugs block morphine-induced feeding. In Experiment 3, it was found that systemic naltrexone blocked the expression of conditioned feeding (confirming Experiment 1), as did SCH-23390,

whereas haloperidol did not affect expression of conditioned feeding. In the fourth experiment, we observed that significant conditioned feeding was induced with repeated treatment with the selective mu agonist D-Ala<sup>2</sup>, NMe-phe<sup>4</sup>, Gly<sup>5</sup>-enkephalin (DAMGO, 2.5  $\mu\text{g}$ ), but not with the delta agonist D-Pen<sup>2,5</sup>-enkephalin (DPEN, 3.1  $\mu\text{g}$ ). The final experiment tested the diurnal variability of the expression of conditioned feeding, and it was found that the magnitude of the effect depended on time of day. In summary, the development of opioid-induced conditioned feeding depends on mu opiate receptor stimulation, but not dopamine receptor stimulation. Its expression, however, involves both opiate and D-1 receptor activation. These findings are considered in terms of putative neural mechanisms governing conditioned meal initiation, and implications for compulsive eating and bulimia are also discussed. [*Neuropsychopharmacology* 23:455–467, 2000] © 2000 American College of Neuropsychopharmacology. Published by Elsevier Science Inc.

**KEY WORDS:** Opiates; Morphine; Conditioning; Dopamine; Ingestive behavior; Food intake

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In the past several decades there has been a great amount of interest in the behavioral function of endogenous opioid peptides. Opioid peptides and their receptors are found in significant concentrations in widespread limbic, forebrain, and brainstem regions, and it is generally believed that these opioid systems play diverse roles in modulating adaptive behavior. One area of research that has received considerable attention is that of ingestive behavior. Morphine and opioid peptides are well established as having potent stimulatory

effects on food intake (Morley et al. 1983; Sanger 1981; Reid 1985; Hoebel 1985; Leibowitz 1985). Moreover, antagonists of opiate receptors reduce food intake (Apfelbaum and Mandenoff 1981; Cleary et al. 1996; Kirkham and Blundell 1987; Ukai and Holtzman 1988).

Although it has long been known that these effects occur in many species, including humans, the precise nature of how opioids modulate food intake has been more difficult to establish. However, one emerging theory that is receiving considerable support is that opioids specifically regulate palatability, that is, the pleasurable or "hedonic" aspects of food stimuli (Cooper and Kirkham 1993; Berridge 1996). There is a variety of support for this notion. First, in human studies, in both normal weight and obese patients, opiate antagonists decrease intake of foods, especially those rich in fat and sugar content, while leaving hunger and satiety ratings unchanged (Fantino et al. 1986; Yeomans et al. 1990; Drewnowski et al. 1992). Naloxone was found to reduce "pleasantness" ratings of the smell and taste of a number of foods, and effects were particularly large in items rated as highly palatable (Yeomans and Wright 1991). Second, animal studies have shown that treatment with opioid antagonists appears to reduce palatability, and conversely, that opioid agonists enhance the pleasantness of food. For example, several earlier studies showed that naloxone or naltrexone reduced preference for sweet taste, and reduced hyperphagia induced by a highly palatable diet (Apfelbaum and Mandenoff 1981; Cooper and Turkish 1989; Le Magnen et al. 1980). Morphine or other selective opioid agonists enhance intake of palatable food, when injected systemically or intracerebroventricularly (Calcagnetti and Reid 1983; Czirr and Reid 1986; Doyle et al. 1993; Gosnell and Majchrzak 1989; Pecina and Berridge 1995).

An important question to consider is where in the brain these effects are being mediated. Although there has been considerable focus on hypothalamic regions (McLean and Hoebel 1983; Stanley et al. 1989; Tepperman and Hirst 1983; Ukai and Holtzman 1988; Woods and Leibowitz 1985), several other regions that are rich in opioid receptors also support opioid-induced feeding, such as amygdala, ventral tegmental area, and ventral striatum (Gosnell 1987).

Recently, our laboratory has focused on the role of opiate receptors within the ventral striatum and their putative role in feeding behavior. Opioid stimulation of the nucleus accumbens and several surrounding ventral striatal sites induced marked enhancement of food intake, with mu receptor stimulation being most potent in this regard (Bakshi and Kelley 1993a,b; Kelley et al. 1996). Moreover, mu opioid stimulation of the nucleus accumbens with the peptide DAMGO selectively enhances sucrose drinking when a choice is given between water and sucrose (Zhang and Kelley 1997), and selectively enhances fat intake when a choice is avail-

able between fat and carbohydrate (Zhang et al. 1998). This profile provides support for the theory that palatability factors in feeding are enhanced by opioids. We have also made the observation that repeated stimulation with morphine in the nucleus accumbens results in sensitized and conditioned feeding; that is, the response to morphine increases progressively with multiple exposures, and when rats are given a sham or saline infusion after repeated treatments, a conditioned feeding response occurs (Bakshi and Kelley 1994). This observation suggests that the environmental stimuli that are associated with the morphine injection (conditioned cues) acquire the ability to elicit a conditioned response. Further, such a phenomenon would suggest the induction of long-term neuroadaptive alterations at the cellular level within the nucleus accumbens or its associated circuits.

There is also a fairly extensive literature showing that contextual signals, such as a particular room where rats are fed, can elicit feeding in sated rats (Calvin et al. 1953; Grant and Milgram 1973; Weingarten 1983; Zamble 1973); moreover, physiological responses such as salivation, insulin secretion, and corticosterone secretion can be readily conditioned to food-associated stimuli (Coover et al. 1977; Sahakian et al. 1981; Woods et al. 1977). Indeed, many comprehensive theories of ingestive behavior postulate that external factors, in addition to internal factors related to energy balance, play an important role in controlling food intake (Robbins and Fray 1980; Toates 1981; Weingarten 1985).

The aim of the present series of experiments was to further investigate the opioid-induced conditioned feeding response, examining pharmacological determinants of the development and expression of the response. In addition to opiate receptor mechanisms, we focused on dopamine (DA) receptors as well, inasmuch as there is considerable evidence for opiate-dopamine interactions in striatal systems (e.g., De Vries et al. 1991; Gerfen et al. 1991; Kalivas and Bronson 1985; Stinus et al. 1992).

## MATERIALS AND METHODS

### Subjects

Male Sprague-Dawley rats (Harlan, Madison, WI) weighing between 275 and 300 g at the time of surgery were used for these experiments. The rats were group-housed with 2–3 rats per cage and were maintained on an *ad libitum* diet of standard laboratory chow, with water available at all times. The animal colony was on a 12:12 hour light/dark schedule with lights on a 7:00 a.m. All testing occurred between 8 a.m. and 5 p.m. Animals were handled following arrival in the laboratory and after surgery in order to adapt them to handling and minimize stress. The care of animals was carried

out in accordance with institutional and international standards of care.

### Surgery

Animals were handled for several days after arrival. For surgery, animals were anesthetized with a mixture of ketamine and xylazine (ketamine, 90 mg/kg; xylazine, 9 mg/kg; Research Biochemicals International, Natick, MA). Bilateral stainless steel guide cannulae (23-gauge, 10 mm) were stereotaxically implanted into the accumbens. Guide cannulae were secured to the skull with stainless steel screws and light curable dental resin (Dental Supply of New England, MA). Coordinates for the aimed site, based on the atlas of Pellegrino and Cushman (1967), were (in mm (with toothbar 5 mm above interaural zero): +3.2 from bregma in the anteroposterior (A-P) plane; 1.5 from midline in the lateromedial (L-M) plane, and -5.6 from skull in the dorsoventral (D-V) plane. After the surgery, wire stylets were placed in the guide cannulae to prevent occlusion. In the present studies, cannulae were not aimed specifically at the core or shell subregions of accumbens. Histological analysis indicated that the injection sites were generally on the border of these subregions.

### Behavioral Testing and Procedure

Behavioral testing occurred between 0800 and 1700 h (8:00 a.m. and 5:00 p.m.), except where time of testing was specified (Experiment 5). Within a given experiment, animals were consistently tested either in the morning or afternoon. Animals were first adapted to the test cages, test room, and infusion procedure by bringing them to the room and placing them in the cages for 2 h immediately after a saline infusion. Rats went through this habituation procedure once or twice prior to testing. The test cages were of clear polycarbonate, with dimensions identical to those of the home cages. Animals were always tested individually. For the adaptation period, food was scattered on the grid floor, and a sheet of paper was placed beneath the grid to catch spillage. A water bottle was available at all times. Although the number of injections and/or test days varied depending on the experiment, all experiments had several common features. There was always a baseline day of testing (measuring food intake) before any opioids were administered. This baseline day was followed by repeated morphine or opiate agonist treatment (between four and six opiate injections). The treatments were given once every two days.

Following the end of treatment, animals were tested for conditioned feeding by repeating the exact procedure as during treatment, except that a sham microinjection was given instead of drug or saline. During this phase, the injectors were lowered, but no infusion was

given. All tests were conducted for 3 h with standard laboratory chow that was weighed before and after testing. Total food intake over 3 h, taking into account any spillage, was the main dependent variable. Consecutive conditioned feeding test days were separated by 1–2 days in which rats received no treatments and remained in the vivarium.

### Drugs and Microinfusions

Morphine was obtained from NIDA; the mu-receptor agonist D-Ala<sup>2</sup>, NMe-phe<sup>4</sup>, Glyol<sup>5</sup>-enkephalin (DAMGO), the dopamine D-1 antagonist SCH 23390, and the opiate antagonist naltrexone were all obtained from Research Biochemicals International (Natick, MA). D-Pen<sup>2</sup>,5-enkephalin (DPEN) was obtained from Bachem (Torrance, CA). DAMGO, morphine, SCH 23390, and naltrexone were dissolved in sterile 0.9% saline. DPEN was dissolved in distilled water. The injectable form of the dopamine D-2 antagonist haloperidol was obtained from Quad Pharmaceuticals (Indianapolis, IN).

For microinjections, which were always bilateral, the stylets were removed first, then the drugs or vehicle in a volume of 0.5  $\mu$ l were infused by lowering 12.5 mm injector cannulae (30 gauge) to the accumbens. Thus, injector tips extended 2.5 mm beyond the end of the guides, for a final D-V coordinate of -8.1 mm from skull. A microdrive pump (Harvard Apparatus), connected via polyethylene tubing (PE-10) was used to infuse the drugs with an injection duration of 2 min followed by a one minute diffusion period. Injectors were then removed, the stylets were replaced and animals were put into the test cage.

### Experimental Design

**Experiment 1: Dose-Response Analysis.** For the first experiment, a total of 32 rats was used. These animals were divided into four groups ( $n = 8$ /group), that received different doses of morphine (0, 1, 5, 10  $\mu$ g/0.5  $\mu$ l; equivalent to 0, 1, 7, 17 nmol, respectively). All groups received baseline testing, five intra-accumbens injections, and one or several conditioning tests depending on the group. The 5- $\mu$ g and 10- $\mu$ g groups received an additional conditioning test day, and the 5- $\mu$ g group underwent an additional conditioning test day with systemic naltrexone pretreatment (5 mg/kg, i.p.). The naltrexone was given ten minutes prior to the test.

**Experiment 2: Determinants of Development of Conditioned Feeding.** This experiment examined whether dopamine D-1 or D-2 receptors were involved in the development of morphine-induced conditioned feeding. There were three groups (total  $n = 24$ ) that were tested as described above. These groups underwent a total of

six intra-accumbens morphine (5 µg/7 nmol) injections, following baseline testing. For all the morphine tests, animals were pretreated with either haloperidol (0.25 mg/kg, i.p., 30 min prior to testing;  $n = 8$ ), SCH 23390 (0.1 mg/kg, i.p., 30 min prior to testing;  $n = 8$ ), or saline (0.3 ml, i.p.; 30 min prior to testing;  $n = 8$ ). Following this regimen, all animals were tested for conditioned feeding. On the conditioned feeding test day, all animals were administered a saline injection 30 min prior to the test in order to mimic the previous test experience.

**Experiment 3: Determinants of Expression of Conditioned Feeding.** The purpose of this experiment was to investigate the role of opiate, D-1, or D-2 receptors in the expression of morphine-induced conditioned feeding. A total of 24 rats was used. These rats received a baseline test day, five intra-accumbens morphine injections, and three conditioning tests following the end of treatment. In the first group ( $n = 8$ ), on the second conditioning test day, rats were pretreated with naltrexone (5 mg/kg, i.p., in order to replicate the results obtained in Experiment 1). The second group ( $n = 8$ ) was pretreated with the D-1 antagonist SCH 23390 on the second test day (0.1 mg/kg, i.p., given 30 min prior to test). The third group ( $n = 8$ ) was pretreated with the D-2 antagonist haloperidol (0.25 mg/kg, i.p., 30 min prior to test) on the second test day. These doses of SCH 23390 and haloperidol have been found previously to potently block dopamine agonist-induced behaviors without causing global effects on locomotor or feeding behaviors, and thus were selected for the present studies (Chu and Kelley 1992; Ettenberg 1990).

**Experiment 4: The Role of Opioid Receptor Subtypes.** In this study, the ability of selective opioid mu or delta agonists to induce conditioned feeding was investigated. Our previous work has shown that morphine, mu, and delta agonists were able to elicit feeding, whereas kappa agonists were inactive. Therefore, in this study, four injections of the mu selective agonist D-Ala<sup>2</sup>,NMe-Phe<sup>4</sup>,Glyol<sup>5</sup>-enkephalin (DAMGO, 2.5 µg;  $n = 8$ ), and four injections of the delta agonist D-Pen<sup>2,5</sup>-enkephalin (DPEN, 3.1 µg;  $n = 8$ ) were administered in the nucleus accumbens.

The doses of each compound chosen, which are equimolar (5 nmol), were the most active based on our previous study. A saline group was also included for this study ( $n = 8$ ). This group underwent baseline testing, four saline injections and a sham/conditioned test day.

**Experiment 5: Diurnal Variability and Expression of Conditioned Feeding.** Over the course of these studies, we had noticed that the magnitude of both morphine-induced feeding and the expression of conditioned feeding was somewhat variable. It appeared that

the effects were somewhat larger if animals were injected with morphine in the afternoon as opposed to the morning. Therefore, an experiment was designed to examine whether the expression of the conditioned feeding effect varied depending on time of day. In one group ( $n = 8$ ), animals underwent baseline testing, intra-accumbens morphine treatment (5 × 5 µg), and the sham injection in the morning. The three hour test began at approximately 8:00 a.m. (0800 h). In the second group ( $n = 8$ ), the animals were tested similarly except that the experiment was carried out in the afternoon, with testing beginning at approximately 1:00 p.m. (1300 h).

### Statistical Analysis

Data were analyzed by multifactorial analysis of variance (ANOVA) using the SuperANOVA program for the MacIntosh (Abacus Concepts). Depending on the experiment, either a between-within model or repeated measures model was utilized. For significant overall interactions, further analysis of partial interactions was carried out. In all experiments, baseline data were compared with the data from the conditioning test days using a repeated measures analysis. Where appropriate, further comparisons between means were carried out by planned contrasts. Since the main focus of these experiments was examination of induction of conditioned feeding, for the most part the drug-induced feeding data were not analyzed, except in Experiment 2.

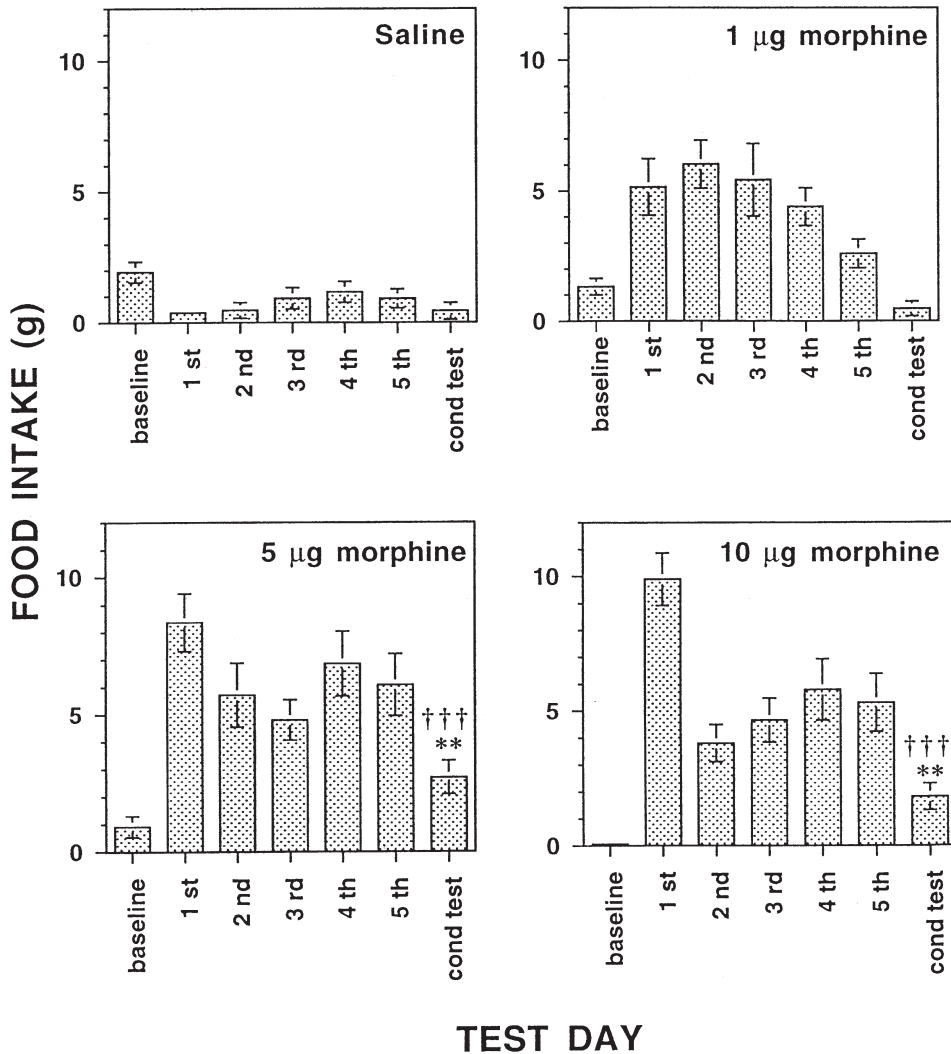
### Histology

At the end of experiments, rats were deeply anesthetized and perfused through the heart with isotonic saline followed by 10% formalin. The brains were removed, post-fixed, and stored in sucrose formalin until sectioning. They were cut into 60 micron sections on a cryostat, and the sections were then stained with cresyl violet and examined under a light microscope for verification of injection placements.

## RESULTS

### Experiment 1

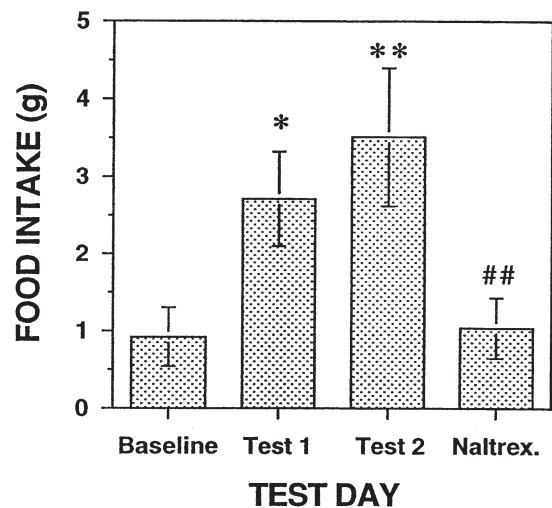
Morphine treatment resulted in a dose-dependent induction of conditioned feeding, as shown in Figure 1. Analysis of variance with dose as the between-subjects factor and test day (baseline vs. sham/conditioning test day) as the within-subjects factor revealed a significant overall dose × test day interaction [ $F(3, 28) = 14.4, p < .001$ ]. Further analysis indicated this effect to be due to a significant interaction between the saline and 5 µg-morphine groups ( $p < .001$ ) and the saline and 10-µg morphine groups ( $p < .001$ ). In Figure 1 it can be ob-



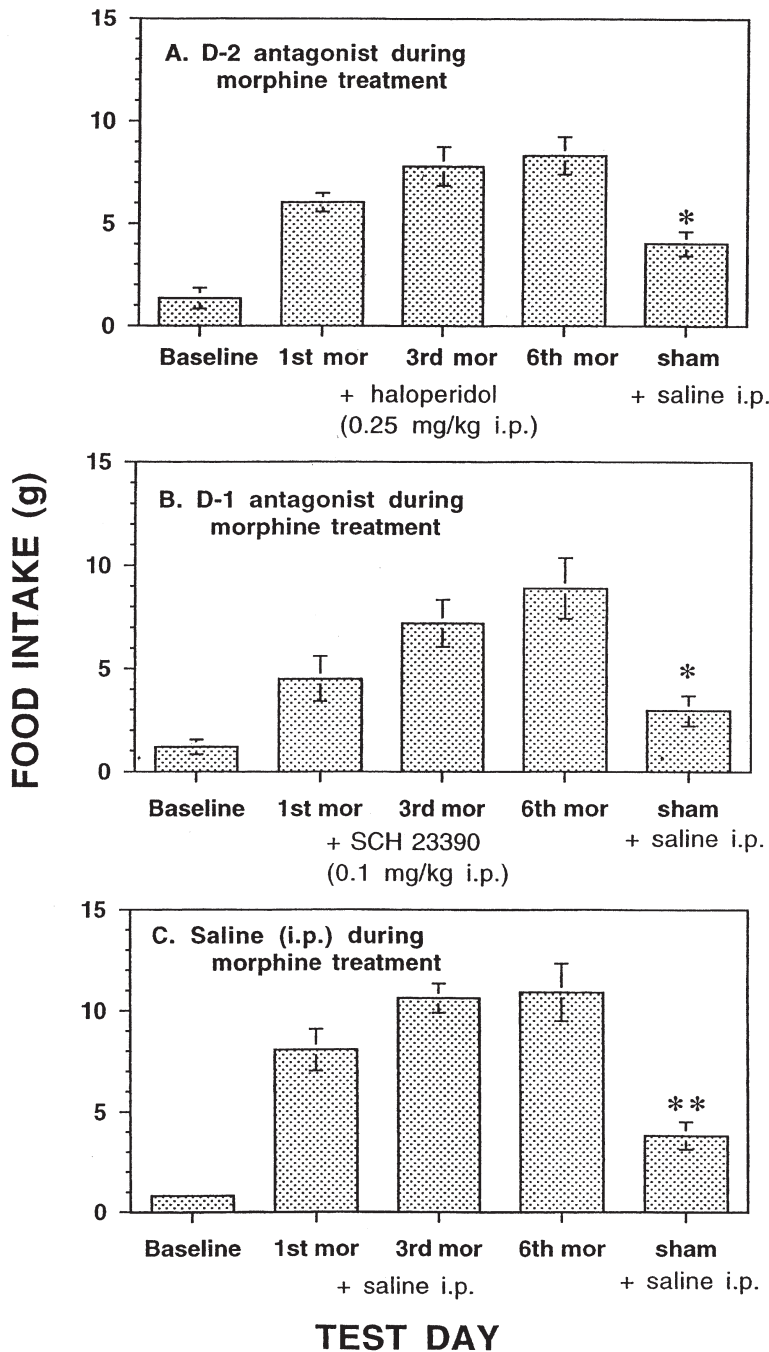
**Figure 1.** Dose-effect curve for induction of conditioned feeding following repeated morphine injections into nucleus accumbens (Experiment 1). Separate groups of animals were administered the different doses. <sup>†††</sup>*p* < .001, significant dose × test day interaction (when comparing baseline days with conditioning test days). Daggers indicate that 5-µg and 10-µg dose groups are significantly different from saline group. <sup>\*\*</sup>*p* < .01, significant difference between baseline and test day within a particular dose group. Bars represent means ± s.e.m., as for all figures.

served that in these two morphine treatment groups, feeding was substantially higher on the conditioning test day than on the baseline day, whereas in the control and low-dose morphine groups, feeding on the test day was either the same or lower than on the initial baseline day.

In the 5-µg group, feeding was even higher on the second conditioning test day (Figure 2). Additionally, this conditioned feeding effect was blocked by prior administration of naltrexone. A repeated-measures ANOVA of the data from these four test days revealed a significant overall effect of test day [*F*(3,21) = 5.37, *p* < .007]. Comparisons between means subsequently indicated significant differences between baseline and test 1 (*p* < .03), baseline and test 2 (*p* < .003), and test 2 and naltrexone (*p* < .004). There was no significant difference between the baseline day and naltrexone day, as is clear from Figure 2.



**Figure 2.** Naltrexone blocks the expression of conditioned feeding (Experiment 1). These data are from the 5-µg morphine treatment group. The dose of naltrexone was 5 mg/kg, i.p. <sup>\*</sup>*p* < .05, <sup>\*\*</sup>*p* < .01, significant difference from baseline. <sup>##</sup>*p* < .01, significant difference from test day 2.



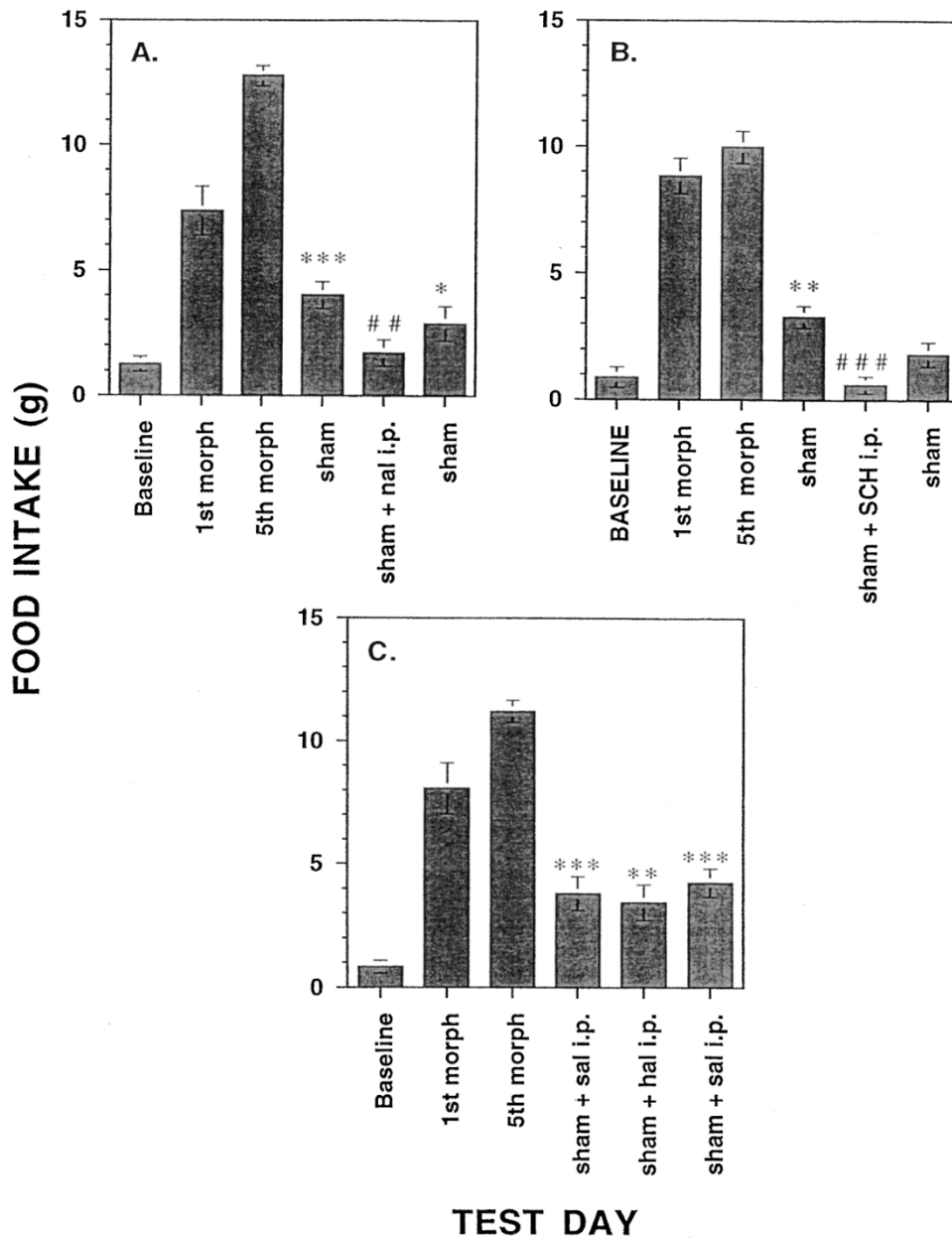
**Figure 3.** Effect of co-treatment with dopamine receptor antagonists on the development of morphine-induced conditioned feeding (Experiment 2). Intra-accumbens morphine treatments (5  $\mu$ g) were given in the presence of either the D-2 antagonist haloperidol (A), the D-1 antagonist SCH 23390 (B), or i.p. saline (C). \* $p < .05$ , \*\* $p < .01$ , significant difference with respect to baseline.

### Experiment 2

The results from examination of the involvement of DA receptors in the development of conditioned feeding are shown in Figure 3. The emergence of a conditioned feeding response following morphine injections was not reduced or blocked by co-treatment with the dopamine antagonists. Analysis of the baseline and sham/conditioning test days for the haloperidol treatment group (Figure 3A) indicated a significant conditioned feeding effect [ $F(1,7) = 7.96, p < .03$ ]. A similar analysis of the SCH 23390 group (Figure 3B) also resulted in a significant effect [ $F(1,7) = 6.00, p < .04$ ], as

was true for the saline treatment group (Figure 3C; [ $F(1,7) = 20.3, p < .01$ ]).

It is also interesting to note that treatment with these antagonists did not greatly reduce the morphine-induced feeding response, which was quite robust even under the influence of neuroleptics. An overall between-within ANOVA of the three treatment groups for the six morphine test days just missed significance [ $F(2,21) = 2.85, p < .08$ ]. However, if the two neuroleptic groups were compared individually with the saline group, the tendency toward a suppressive effect on morphine-induced feeding reached significance [ $F(1,14)$



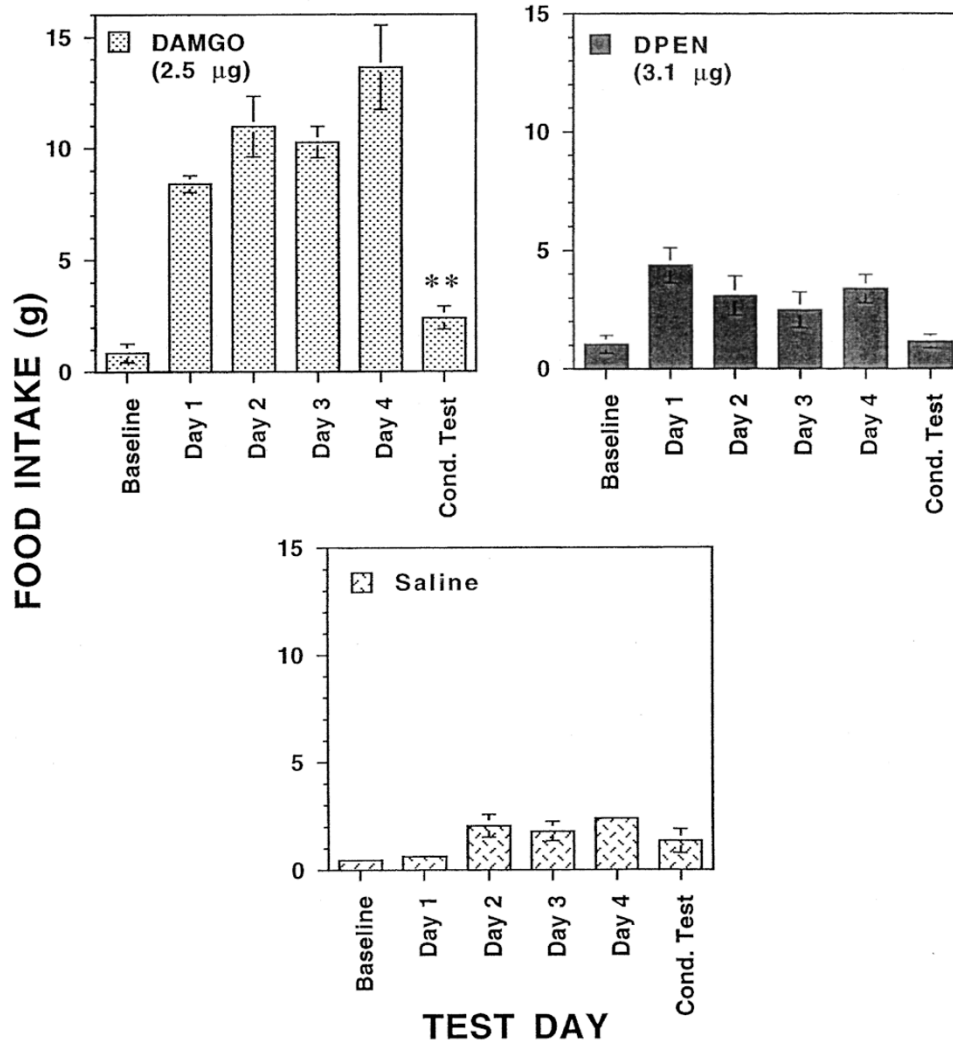
**Figure 4.** Expression of conditioned feeding following repeated morphine treatment in nucleus accumbens (Experiment 3). Effect of naltrexone (nal; 5 mg/kg, A), SCH 23390 (SCH; 0.1 mg/kg, B) or haloperidol (hal; 0.25 mg/kg, C) on expression of conditioned feeding. \* $p < .05$ , \*\* $p < .01$ , \*\*\* $p < .001$ , significant difference with respect to baseline control. ## $p < .01$ , ### $p < .001$ , significant difference with respect to previous sham conditioning day.

$p < .02$ ] for the haloperidol group, and still just missed significance for the SCH 23390 group [ $F(1,14) = 3.96$ ,  $p < .066$ ]. These analyses suggest that treatment with DA antagonists tended to reduce intra-accumbens morphine-induced feeding, but the effect was not robust.

**Experiment 3**

Figure 4 shows that the expression of the conditioned feeding response elicited by prior treatment with mor-

phine was blocked by co-treatment with naltrexone and SCH 23390, but not with haloperidol. For the experiment with naltrexone, a significant overall effect of test day was found [ $F(3,21) = 6.35$ ,  $p < .003$ ]. Further analysis indicated a difference between baseline and the first conditioning test ( $p < .001$ ), between baseline and the second conditioning test ( $p < .03$ ), and between the first conditioning test and naltrexone ( $p < .003$ ), as shown in Figure 4A. There was no difference between naltrexone and baseline. A similar profile was found for the experiment with SCH 23390 (Figure 4B).



**Figure 5.** Conditioned feeding following repeated intra-accumbens infusions of either the mu-selective peptide DAMGO or the delta-selective peptide DPEN, or saline (Experiment 4).  $**p < .01$ , significant difference compared with baseline.

Comparisons following the significant overall ANOVA [ $F(3,21) = 7.22, p < .002$ ] revealed a difference between baseline and the first conditioning test ( $p < .001$ ), and between the first conditioning test and SCH 23390 ( $p < .001$ ). There was no difference between baseline and SCH 23390. In this experiment, conditioned feeding was weaker on the second test, with the level not being significantly different from baseline. For the experiment with haloperidol, there was a significant overall effect of test day [ $F(3,21) = 7.9, p < .001$ ]. Further analysis demonstrated a significant difference between baseline and all three test days, as shown in Figure 4C.

#### Experiment 4

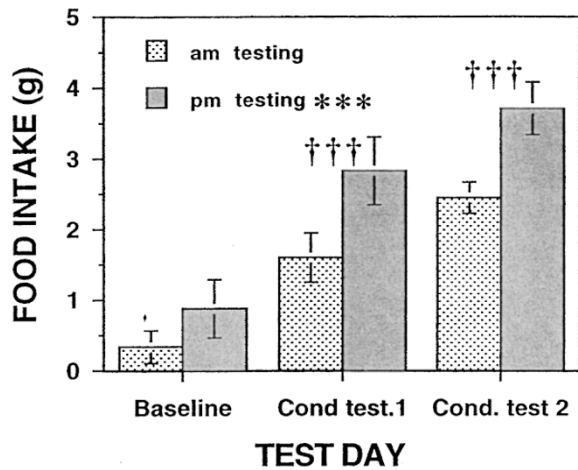
As shown in Figure 5, previous repeated intra-accumbens treatment with the mu agonist DAMGO induced a significant conditioned feeding effect [ $F(1,7) = 10.8, p <$

.01]; moreover, as reported in our previous work (Bakshi and Kelley 1993b), DAMGO induced a marked increase in food intake. Equimolar injection of the delta agonist DPEN induced a smaller enhancement of food intake, also in accord with the previous study. However in this group, a conditioned feeding effect was not observed. No significant effects were found for the saline control group, as expected.

#### Experiment 5

Expression of the conditioned feeding effect induced by morphine appeared to be stronger if the tests were administered in the afternoon rather than the morning (Figure 6). The morphine-induced feeding also tended to be higher in the later test period, although this tendency did not reach significance (results not shown). However, the difference for expression of conditioned





**Figure 6.** Conditioned feeding magnitude depends on time of day (Experiment 5). Animals were given four intra-accumbens morphine infusions (5  $\mu$ g) between baseline and conditioned test days  $***p < .001$ , main effect of group (time of day),  $†††p < .001$ , main effect of test day: either conditioned feeding test is significantly different from baseline test day.

feeding was quite marked. Analysis of these data indicated a main effect of group [ $F(1,14) = 10.26, p < .006$ ], and of test day [ $F(2,28) = 27.2, p < .001$ ]. This profile indicates that as a group, the animals tested in the afternoon showed a greater feeding response than those tested in the morning. Additionally, feeding on the first and second conditioning test days was significantly greater than on the baseline day ( $p < .001$ ). It is also interesting to note that feeding on conditioning test day 2 was greater than on test day 1, as was also found in Experiment 1.

## DISCUSSION

The findings described above confirm our previous initial demonstration that animals previously treated with intra-accumbens morphine and allowed to feed show a conditioned feeding response when exposed to the same environment where they received morphine-feeding pairings (Bakshi and Kelley 1994). Several further conclusions can be drawn from these experiments. First, the development of conditioned feeding is dose-dependent; only higher morphine doses, that caused robust feeding, resulted in a conditioned feeding response. Second, development of conditioned feeding appears to depend on mu opiate receptor activation. Additionally, co-treatment with DA antagonists during the morphine treatments did not block the unconditioned morphine-induced feeding response, nor the development of a conditioned feeding response. Third, once established, the expression of conditioned feeding

is clearly dependent on activation of brain opiate receptors, as well as activation of dopamine D-1 receptors, but not D-2 receptors. These results can be discussed in terms of both neural and behavioral mechanisms.

The dose-response study revealed several interesting features of opiate-induced conditioned feeding. It appears that there is a threshold dose for development of the conditioned feeding response, somewhere in the range of 1–5  $\mu$ g. The lower dose of morphine, 1  $\mu$ g, induced a fairly robust feeding response but yet in these rats, conditioned feeding did not develop. This situation suggests that repeated experience with morphine-feeding pairings *per se* is not sufficient to induce conditioned feeding. This notion is supported by the fact that intra-accumbens treatment with the delta-selective peptide DPEN also enhances chow feeding, as shown in Figure 5 and in our previous work (Bakshi and Kelley 1993b), but also does not result in a conditioned response. Thus, the neural or cellular alterations that support conditioned feeding may require the presence of high synaptic concentrations of opioids. Dose-dependency for the induction of morphine sensitization for locomotor activity has also been demonstrated (Bartoletti et al. 1987).

It should be noted at the outset that although the conditioned response is highly replicable from experiment to experiment, its magnitude is substantially lower than the opiate-induced feeding response. This is not surprising in that conditioned responses are often weaker in strength than the corresponding unconditioned responses. We have also shown that its duration is considerably shorter than the drug-induced response (Bakshi and Kelley 1994). It is important to note that for the most part, conditioned feeding is significant in relation to both the previous within-subjects baseline, and to a saline-treated (between subjects) control. However, there is some obvious variability in the feeding responses, particularly with regard to baseline. For example, in Figure 1 it can be observed that initial mean baseline food intake was nearly absent in the 10  $\mu$ g group, whereas it was somewhat higher in the saline group. In general, however, baseline food intake amongst groups was comparable. It remains to be seen in future studies what influence (if any) circadian rhythms have on opiate-induced conditioned feeding. In the present studies, it was found that testing animals in the afternoon versus the early morning resulted in a higher level of baseline and conditioned feeding. However, in both sets of animals, a robust conditioned feeding response was noted, suggesting that the conditioned feeding effect is not simply a function of time of day.

It was shown in both Experiments 1 and 3 that the expression of conditioned feeding is blocked by co-treatment with the opiate antagonist naltrexone. This result suggests that the contextual cues that elicit feed-

ing involve an opiate receptor mechanism. Exposure to the drug-paired environment may elicit similar interoceptive cues as the opioid treatment, albeit less salient in strength. These interoceptive cues may be correlated with either enhanced endogenous opioid peptide release, enhanced sensitivity of opiate receptors, or perhaps, alterations in intracellular signal transduction events (see below). Thus, it may be that opiate receptors are an important component of the final common pathway for ingestive behavior that is activated by the contextual cues. While it is likely that these putative neural events take place within the accumbens, since this is the region that was stimulated, involvement of opioids in other regions cannot be ruled out. An experiment with intracerebral infusions of opiate antagonists in the accumbens and other candidate regions, in combination with conditioned feeding, would address this question. An additional study that supports the involvement of endogenous opioids in conditioned feeding was conducted by Weingarten and Martin (1989). In animals that were classically conditioned to initiate feeding in response to a conditioned stimulus paired with food, prior administration of systemic naloxone reduced conditioned feeding. These authors further found that naloxone did not block food-anticipatory behaviors; conversely, administration of a dopamine antagonist did not affect amount consumed during conditioned feeding, but did block anticipatory behaviors associated with presentation of the conditioned stimulus.

The D-1 antagonist SCH 23390 also appeared to block expression of conditioned feeding, whereas co-administration of the D-2 antagonist did not affect the feeding response. The blockade is unlikely to be due to a motoric inhibition, since animals were capable of morphine-induced feeding under the D-1 antagonist treatment, and since in pilot experiments these doses of antagonists did not inhibit free-feeding in hungry rats (unpublished findings). Rather, it is likely that the opiate-mediated expression of conditioned feeding is dependent on co-activation of D-1 receptors within the ventral striatum. In this regard, it is of interest to note that a taste that was paired with intragastric infusion of food was able to elicit enhanced dopamine release within the nucleus accumbens (Mark et al. 1994). Moreover, there are a number of interesting findings in the literature suggesting a significant interaction between mu opioids and D-1 receptors. For example, place conditioning induced by morphine is blocked by D-1 antagonists, but not D-2 antagonists (Shippenberg and Herz 1987; Shippenberg et al. 1993).

Several investigations have implicated DA, and particularly D-1 receptor function, in chronic effects of morphine and in behavioral sensitization. D-1 antagonists are able to block the expression of morphine-induced sensitization of locomotor activity and stereotypy (Pollock and Kornetsky 1989; Jeziorski and White

1995; Livezey et al. 1995), and intermittent exposure to morphine causes cross-sensitization to amphetamine (Cunningham et al. 1997; Cunningham and Kelley 1992). D-1-opioid interactions at the level of signal transduction and gene expression may underlie these behavioral effects. In chronically morphine-treated animals, there is an upregulation of D-1 stimulated cyclic AMP production, with no concomitant increase in mu receptor binding (De Vries et al. 1991, 1993).

In terms of gene regulation, it has been shown that D-1 receptor tone plays a critical role in regulating both basal and stimulant-induced proenkephalin gene expression (Angulo and McEwen 1994; Wang and McGinty 1996, 1997). Further, immediate early gene expression in the striatum induced by morphine is dependent on D-1 receptor activation. Taken together, these findings suggest that the long-term behavioral and neuromolecular alterations induced by repeated exposure to opiates may involve interactions between mu and D-1 receptors systems at the intracellular level.

Considering the development of conditioned feeding, we have demonstrated that multiple intra-accumbens infusions of the mu-selective peptide DAMGO, but not the delta-selective peptide DPEN, resulted in conditioned feeding. Since morphine itself preferentially activates mu receptors, and since the acute DAMGO-induced feeding response is completely blocked by naltrexone (Bakshi and Kelley 1993b; Zhang et al. 1998), it is likely that the *induction* of conditioned feeding is dependent on activation of mu opiate receptors. In contrast, neither D-1 nor D-2 receptors appear to play a key role in the development of the effect, since animals treated with intra-accumbens morphine showed strong feeding responses even in the presence of acute neuroleptics, and showed conditioned feeding when given the sham intra-accumbens injection. It should also be noted that while kappa receptors are found abundantly in the ventral striatum (Mansour et al. 1987), their stimulation in this region does not alter food intake (Bakshi and Kelley 1993b; Majeed et al. 1986; Zhang and Kelley 1997). It would be interesting to know if glutamate receptors are involved in the induction of conditioned feeding, since co-treatment with an NMDA antagonist blocks development of morphine locomotor sensitization (Jeziorski et al. 1994). This possibility awaits further study.

Many previous reports have described the phenomenon of context-dependent conditioned feeding (Calvin et al. 1953; Grant and Milgram 1973; Weingarten 1983; Zamble 1973), and it is well established in both human and animal studies that the physiological and behavioral effects of opiates can become conditioned to environmental stimuli associated with drug exposure (Childress et al. 1986; Davis and Smith 1976; Siegel 1976; Stewart et al. 1984). Moreover, while some of this work suggests a so-called "anti-opiate" conditioning re-

sponse (e.g., Siegel 1976), there is also evidence for conditioned opioid release in the presence of opiate-specific cues (Davis and Smith 1974; Fanselow 1982; Kehoe and Blass 1989; Weingarten and Martin 1989). Traditional learning theory posits that stimuli associated with food (appetitive conditioned stimuli) acquire motivating or incentive properties through repeated pairings (Bindra 1968; Toates 1981). Additionally, these stimuli are able to elicit secretory-motor responses ("cephalic phase" responses, such as salivation, insulin secretion) that may prepare the organism to eat (Weingarten 1985). The present study suggests one possible mechanism for the central mediation of learned stimulus control of feeding behavior. Sensory cues associated with the feeding environment could activate opioid release within the accumbens, resulting in initiation of feeding. A putative substrate for this mechanism would be the glutamate input from corticolimbic regions; cortical afferents directly synapse onto the enkephalin-containing striatum medium-spiny output neurons (Bouyer et al. 1984; Smith and Bolam 1990).

Regarding the clinical relevance of this work, binge eating observed in eating disorders such as bulimia nearly always involves highly palatable food (e.g., high sugar/fat). Moreover, since central opioids are implicated in the hedonic response to high palatability of foods (Berridge 1996; Cooper and Kirkham 1993; Fantino et al. 1986), one could also speculate that food cravings and compulsive eating in response to certain cues or contexts may involve the nucleus accumbens. Our recent work shows that mu opioid receptor stimulation in this region results in marked, and specific, increases in sugar and fat intake (Zhang et al. 1998; Zhang and Kelley 1997). It has been suggested that conditioning processes are important in the establishment of disturbed eating patterns, and may also be relevant to their treatment (Wardle 1990). Thus, the neural alterations associated with repeated opioid activation of the nucleus accumbens may in part be involved in the process whereby learned cues influence excessive eating.

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