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# NUCLEUS ACCUMBENS OPIOIDS REGULATE FLAVOR-BASED PREFERENCES IN FOOD CONSUMPTION

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**Abstract—Opioid signaling in the nucleus accumbens (NAcc) regulates feeding behavior, having particularly strong effects on consumption of highly palatable foods. Since macronutrient content may contribute to palatability, it is uncertain whether opioid regulation of food consumption is based primarily on its macronutrient content or its flavor per se. In order to isolate the effect of flavor, we manipulated opioid signaling in the NAcc in rats and quantified consumption of differently flavored but nutritionally identical pellets. When pellets of either flavor were presented alone, microinjection of D-Ala$^2$,N,Me-Phe$^4$,Gly-ol$^5$-enkephalin (DAMGO (a $\mu$ opioid receptor (MOP) agonist)) into the NAcc increased consumption of pellets of both flavors equally. When both flavors of pellets were presented simultaneously, however, DAMGO in the NAcc selectively increased, while naltrexone (a non-selective opioid antagonist) in the NAcc selectively decreased, consumption of the more preferred flavor. Systemic naltrexone injection had no flavor specific effects, decreasing consumption of both flavors equally. Non-selective inactivation of NAcc neurons by local microinjection of muscimol (a $GABA_A$ agonist) increased consumption of both the more- and less-preferred flavors equally. These results indicate that opioid signaling directly regulates a subset of NAcc neurons that can selectively enhance consumption of preferred palatable foods based exclusively on flavor cues. © 2006 IBRO. Published by Elsevier Ltd. All rights reserved.**

**Key words: opioids, nucleus accumbens, feeding, palatability, choice.**

Flavor is one of the orosensory qualities of food that determines its hedonic value, or palatability, and is an important determinant of what and how much we eat. Among the molecular signaling systems that regulate palatability are the opioid peptides and receptors. Opioid agonists induce robust feeding in the rat (Martin et al., 1963; Kelley et al., 2002) by increasing the consumption of palatable food (Calcagnetti and Reid, 1983; Berridge, 1996) and microdialysis experiments indicate that palatable foods stimulate release of endogenous opioids in the hypothalamus (Dum et al., 1983). Importantly, opioid antagonists decrease preference for sweet foods in rats without affecting chow or water intake (Cooper, 1983). Furthermore, the "taste reactivity test" (a test that examines the orofacial-affective responses of the rat) indicates that morphine enhances (Doyle et al., 1993) while general opioid antagonists decrease, the positive hedonic effects of palatable food consumption (Parker et al., 1992). Consistent with this interpretation, human subjects given the opioid antagonist naltrexone report that food does not taste as delicious, although taste intensity and recognition thresholds are not affected (Yeomans and Gray, 2002). In fact, none of these opioid effects on consumption are associated with a change in the ability to discriminate tastes (O'Hare et al., 1997).

The nucleus accumbens (NAcc) is a critical site for opioid regulation of palatability. Microinjection of D-Ala$^2$,N,Me-Phe$^4$,Gly-ol$^5$-enkephalin (DAMGO) (a $\mu$ opioid (MOP) receptor selective agonist) into the NAcc selectively increases consumption of calorie dense (sucrose and lard (Zhang et al., 1998)) and flavorful (saccharin and salt (Zhang and Kelley 2002)) palatable items while leaving consumption of simultaneously available chow and water unchanged (Kelley et al., 2002). However, DAMGO potently increases chow consumption if it is the only food available (Ragnauth et al., 2000). Furthermore, opioid antagonists injected into the NAcc can block consumption of palatable foods, indicating that endogenous opioid release modulates feeding at this site (Bodnar et al., 1995).

There is evidence that NAcc neurons exert a predominantly inhibitory effect on feeding. Within the shell region of the NAcc, blockade of glutamate receptors increases, while activation of glutamate receptors decreases feeding (Maldonado-Irizarry et al., 1995; Stratford et al., 1998) (but see (Echo et al., 2001)). Furthermore, both $GABA_A$ (muscimol) and $GABA_B$ (baclofen) agonists elicit robust, dose-related increases in chow intake when microinjected into the shell but not the core of the NAcc. Additionally, increasing levels of endogenous GABA by blocking GABA breakdown increases feeding (Stratford and Kelley, 1997). These effects are specific to food; gnawing and water consumption are unaffected. It is important to point out, however, that there are important differences between GABA and opioid induced feeding; while DAMGO-induced feeding is palatability and macronutrient specific, GABA-induced feeding is not (Basso and Kelley, 1999).

Many studies have shown increases in fat consumption with systemic morphine administration (for review see (Zhang et al., 1998)). However, it is not clear that fat content is the critical variable since foods with high fat content are also among the most palatable. For example, systemic morphine increases consumption of a food pre-

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*Abbreviations:* ANOVA, analysis of variance; DAMGO, D-Ala$^2$,N,Me-Phe$^4$,Gly-ol$^5$-enkephalin; MOP, $\mu$ opioid; NAcc, nucleus accumbens.


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viously determined to be preferred irrespective of its macronutrient content (Gosnell et al., 1990). Intra-NAcc DAMGO, however, increases fat consumption irrespective of baseline preference (Zhang et al., 1998) suggesting that opioid stimulation in the NAcc is selective for the macronutrient fat. However, consumption of highly palatable noncaloric solutions like saccharin are also increased by intra-NAcc opioid stimulation. Therefore, opioid-enhanced feeding must be at least partially related to the flavor of a particular food. The present study was specifically designed to investigate the relationship between NAcc opioid manipulation and taste preference. By using differently flavored versions of the same food item, macronutrient composition can be held constant while taste-determined palatability is systematically varied.

## EXPERIMENTAL PROCEDURES

All procedures were approved by the University of California, San Francisco, Animal Care and Use Committee. Every attempt was made to minimize the number of animals used and their suffering.

### Animals

A total of 65 male rats (Long Evans, Charles River Laboratories, Wilmington, MA, USA) weighing between 270 and 480 g were used in the present studies. Animals were individually housed in conventional hanging cages in a temperature- and humidity-controlled room on a 12-h light/dark cycle. Animals had *ad libitum* access to water at all times and *ad libitum* access to chow at all times except during testing.

### Surgery

Animals were anesthetized with isoflurane, their heads placed in a stereotaxic device and then following a small craniotomy, bilateral guide cannulae were stereotaxically placed and then secured to the skull with stainless steel screws and dental cement. Coordinates for the target sites were 1.5 mm anterior, 1.1 mm lateral and 5.5 mm ventral from Bregma. For this study, the cannulae were not directed specifically at the core or the shell regions of the NAcc. Animals were allowed 4 days' recovery postsurgery.

### Drugs and injections

For microinjections, DAMGO, the MOP selective agonist, naltrexone, a non-selective opioid receptor antagonist, and muscimol, the selective $GABA_A$ receptor agonist, were obtained from Sigma Pharmaceuticals (St. Louis, MO, USA). All of these drugs were dissolved in 0.9% sterile saline (for DAMGO 0.25 $\mu$g per side, for naltrexone 20 $\mu$g per side, for muscimol 50 ng per side). These doses were chosen because they have been shown to be effective in altering consumption when injected into the NAcc (Bodnar et al., 1995; Stratford and Kelley 1997; Zhang et al., 1998). First, the stylet was removed from the guide cannulae and the injector cannulae were inserted. The injector cannulae protruded 2 mm past the end of the guide cannulae for a final distance of 7.5 mm ventral to Bregma. The drugs, in a volume of 0.5 $\mu$l of saline, were infused through injector cannulae connected to a microdrive pump by polyethylene tubing. The rate of infusion was 0.25 $\mu$l/min. The injector cannulae remained in place an additional minute after the infusion in order to allow for diffusion. Injectors were then removed and the stylets were replaced. For s.c. injections, naltrexone was diluted in 0.9% sterile saline at a concentration of 1 mg/kg for naltrexone and injected s.c. with a 1 ml syringe. This concentration was chosen because it has been shown to reduce consumption (Cooper, 1980).

### Behavioral testing and experimental design

After recovery from surgery (four days), animals were extensively handled. In order to overcome taste neophobia, rats were brought into the testing room on four separate days and given one hour simultaneous access to both flavors of pellets (chocolate and banana). After this initial exposure, all rats avidly consumed the pellets when available. The two types of flavored 1 g pellets were made from the same meal substrate and were thus matched for all macro- and micronutrients (Bio-Serv, Frenchtown, NJ, USA). Pellets were always delivered in test tube dispensers. Rats were required to bite the pellets and pull them from a hole in the bottom of the tube. This level of effort encouraged the rats to only take what they would eat, hence, rats seldom consumed less than a full pellet greatly facilitating consumption quantification. Every 15 minutes postinjection, the number of pellets remaining in the dispenser was counted and a visual inspection of the cage for dropped pellets was made. Each experiment utilized a new set of rats except where noted. All behavioral experiments occurred during the light phase. The animals had *ad libitum* access to standard rat chow and water when not being tested. There was at least a 48 h interval between microinjections. When a choice was available, the side on which each flavor was presented was randomized each session. When no choice was available, the pellets were placed centrally on the cage wall.

### Data analysis

All data are expressed as mean$\pm$S.E.M. Data were analyzed using repeated measures analysis of variance (ANOVA) with pharmacologic manipulation and flavor as within subject factors. Post hoc comparisons were made using the Student-Newman-Keuls method.

### Histology

After the completion of testing, rats were deeply anesthetized with sodium pentobarbital (390 mg/kg) and transcardially perfused with a 0.9% isotonic saline solution followed by 10% formalin solution. Brains were removed and stored in 10% formalin for several days followed by an overnight immersion in 10% sucrose solution. Brains were sliced into 45 $\mu$m sections, mounted and stained with a Neutral Red stain. Sections were examined under the microscope in order to determine placement of microinjector tips.

## RESULTS

### Baseline flavor preferences

Rats showed a significant preference for chocolate pellets over banana pellets. To determine baseline flavor preferences, animals ($n=14$) were given five 1.5 h sessions of simultaneous *ad libitum* access to both chocolate and banana pellets. This testing occurred after the standard sessions of flavor exposure to overcome taste neophobia that all rats in these experiments received. Sessions were separated by at least 48 h. The average consumption of chocolate pellets was significantly higher than banana ($3.83\pm0.31$ vs. $2.50\pm0.34$, $P<0.05$). When individual preference scores were calculated (banana consumption divided by total consumption averaged across all five testing days), only three animals showed a significant preference for chocolate over