Neuroscience 143 (2006) 309–317

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 μ 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 μ 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, μ opioid; NAcc, nucleus accumbens.

doi:10.1016/j.neuroscience.2006.06.067

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 μg per side, for naltrexone 20 μg 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 μl of saline, were infused through injector cannulae connected to a microdrive pump by polyethylene tubing. The rate of infusion was 0.25 μl/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 μm 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 ± 0.31 vs. 2.50 ± 0.34, $P<0.05$). 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

Table 1. Mean flavor preferences for 14 rats over five testing sessions

Rat	Banana preference
#1	54±14.9%
#2	68±17.5%
#3	31±19.2%
#4	38±12.9%
#5	43±16.3%
#6	51±6.4%
#7	34±11.0%
#8	19±14.5%
#9	23±13.2%
#10	43±6.8%
#11	18±9.7%
#12	31±10.9%
#13	40±19.8%
#14	39±14.4%
Average	38±3.7%

Data expressed as total banana pellets consumed divided by total pellet consumption±standard error.

banana pellets when analyzed individually. However, there was an overall significant group preference for chocolate (38%, C.I. ±7.3%). No animal showed a significant preference for banana over chocolate pellets as determined by confidence intervals. (Table 1).

Intra-NAcc microinjection of DAMGO enhances consumption of each flavor when presented separately

Animals (n=14) were microinjected with DAMGO or saline into the NAcc and then given 1.5 h *ad libitum* access to either banana or chocolate pellets. All rats underwent all four conditions and individual rats were randomly assigned to groups randomized for the order of injection and flavor. DAMGO in the NAcc increased consumption of either flavor when presented alone. Repeated measures ANOVA indicates that DAMGO significantly increased consumption [$F(1,13)=21.548$, $P<0.001$] (Fig. 1a, b) with no significant drug×flavor interaction. Further analysis indicated that these differences are significant from the 60 min time point onward. Despite the baseline preference of rats for chocolate pellets, in the absence of choice, there were no differences in consumption between chocolate or banana pellets following either saline or DAMGO microinfusion.

Intra-NAcc microinjection of a MOP selective agonist selectively enhances consumption of a preferred flavor when given in a choice paradigm

To determine whether DAMGO affects flavor consumption differently when rats are allowed to choose between flavors, rats (n=13) were microinjected with DAMGO or saline and given 1.5 h simultaneous *ad libitum* access to both chocolate- and banana-flavored pellets. All rats underwent both conditions and injection order was randomized.

Intra-NAcc DAMGO increases chocolate consumption when the alternative flavor is banana. Repeated measures ANOVA indicated that DAMGO significantly increased consumption [$F(1,12)=27.680$, $P<0.001$] and there was a significant drug×flavor interaction [$F(1,12)=5.377$, $P<0.05$] (Fig. 2a). Further analysis indicated that these differences were significant from the 30 min time point onward. Post hoc mean contrasts conducted on data from the 90 min time point indicated that rats ate significantly more chocolate than banana pellets with DAMGO ($P<0.001$). This finding was due to a significant DAMGO-induced increase in chocolate consumption ($P<0.001$) (Fig. 2b). In contrast to the large increase produced by DAMGO in consumption of banana when it is the only taste available,

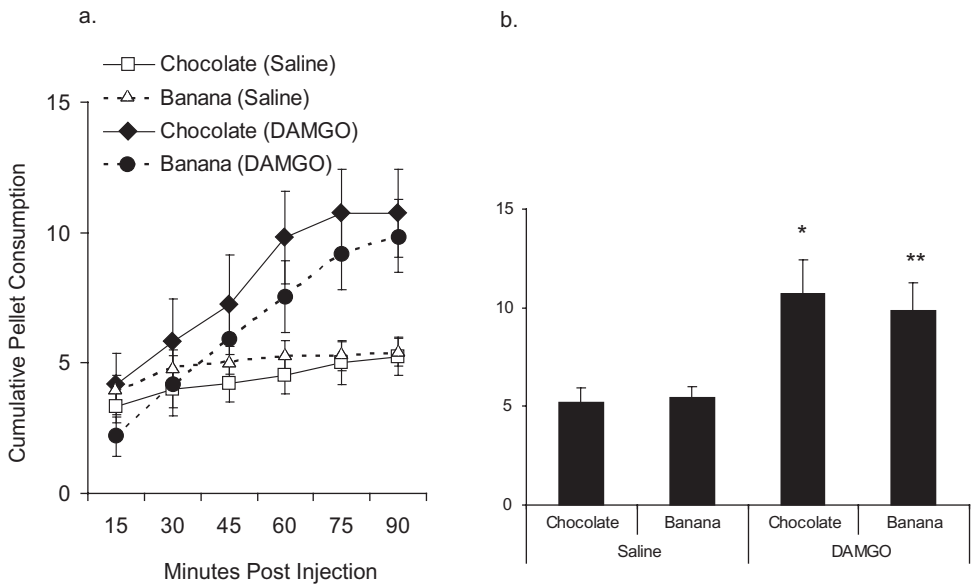

Fig. 1. Effect of intra-NAcc MOP receptor agonist on consumption in the absence of choice. (a) The cumulative number of flavored pellets consumed following saline or DAMGO microinjection is shown for each 15 min postinjection. (b) Cumulative consumption at the 90 min time point. * Indicates $P<0.05$, ** indicates $P<0.01$ compared with saline condition for the same flavor.

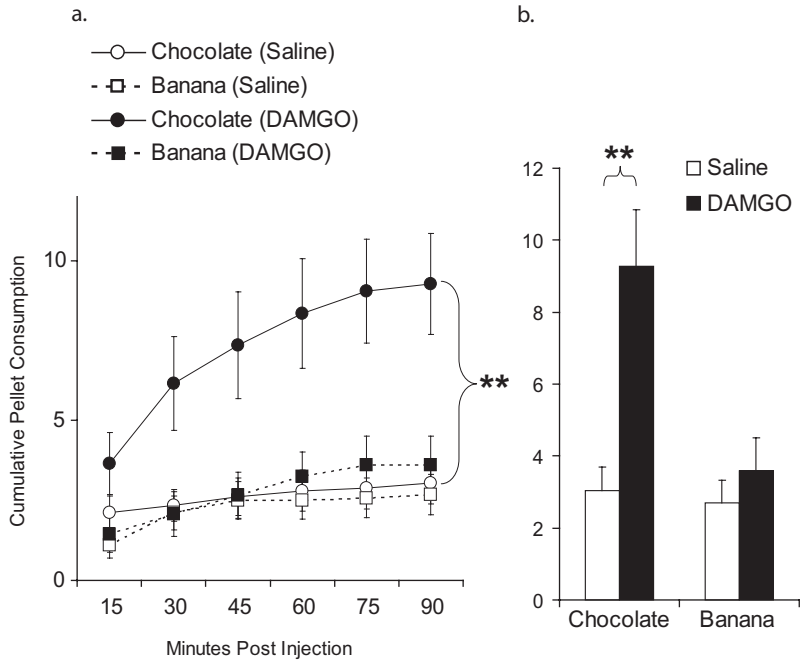

Fig. 2. Effect of intra-NAcc MOP receptor agonist on consumption in a choice paradigm. (a) The number of flavored pellets consumed following saline or DAMGO microinjection is shown for each 15 min postinjection. Since both flavors were available after microinjection, filled symbols represent data from the saline test session while open symbols represent data from the DAMGO test session. (b) Cumulative consumption at the 90 min time point.

in the choice paradigm with chocolate as the alternative, there was no significant DAMGO-induced increase in banana pellet consumption.

Intra-NAcc microinjection of the non-selective opioid antagonist naltrexone

To determine whether intra-NAcc naltrexone affects flavor consumption when rats are allowed to choose between flavors, rats ($n=20$) underwent a choice paradigm identical to the one used previously except they were microinjected with naltrexone (instead of DAMGO) or saline and given 1.5 h simultaneous *ad libitum* access to both chocolate and banana flavored pellets. All rats underwent both conditions and injection and flavor orders were randomized. Intra-NAcc naltrexone selectively decreased consumption of chocolate-flavored pellets when the alternative flavor is banana. Repeated measures ANOVA indicated that naltrexone significantly decreased consumption [$F(1,19)=4.051$, $P<0.05$] and there was a significant drug×flavor interaction [$F(1,19)=4.748$ $P<0.05$] (Fig. 3a). Further analysis indicated that these effects were significant only for the 90 min time point although there was a trend for the interaction after 75 min. The significant drug×flavor interaction was due to a significant naltrexone-induced decrease in consumption of the chocolate-flavored pellets ($P<0.05$) (Fig. 3b). Thus, naltrexone selectively decreases consumption of pellets with the more preferred chocolate flavor.

Systemic naltrexone and intra-NAcc muscimol in the choice paradigm

To compare the effects of naltrexone delivered systemically to those of naltrexone delivered directly into the NAcc, naltrexone injections were made s.c. ($n=24$). Unlike intra-NAcc naltrexone, systemic naltrexone decreased consumption of both flavors equally. Repeated measures ANOVA indicated that naltrexone significantly decreased consumption [$F(1,23)=15.1414$, $P<0.001$] (Fig. 4). There was no significant drug×flavor interaction. Further repeated measures ANOVAs indicate that the significant effect of systemic naltrexone on consumption emerged by 15 min. Post hoc mean contrasts performed on the 90 min time point indicated that there was a significant difference in consumption between saline and naltrexone conditions for both chocolate and banana pellets ($P<0.05$).

To determine the specificity of the NAcc opioid effect on flavor preference, NAcc neurons were inhibited by microinjecting the GABA-A receptor agonist muscimol in the choice paradigm. Rats ($n=17$) were microinjected with muscimol or saline and given 1.5 h simultaneous *ad libitum* access to both chocolate- and banana-flavored pellets. Eight of these rats were used previously in the intra-NAcc naltrexone experiment. The consumption patterns were not different between these two groups so their data were combined and analyzed together. All rats underwent both conditions and injection and flavor orders were randomized. Unlike intra-NAcc DAMGO, intra-NAcc muscimol increased consumption of both chocolate and banana pellets equally when they were presented simultaneously. Repeated measures ANOVA on the 90 min time point showed a significant muscimol induced increase in feeding [$F(1,16)=11.151$, $P<0.005$] (Fig. 5a, b). There was no significant drug×flavor interaction. Further ANOVAs indicated that the significant drug effect emerged by 15 min

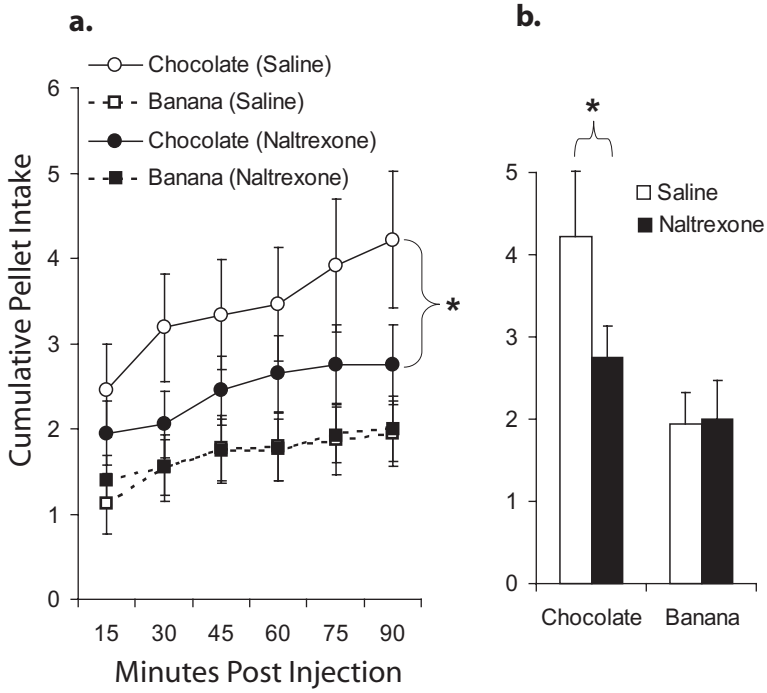

Fig. 3. Effect of intra-NAcc opioid receptor antagonist on consumption in a choice paradigm. (a) The number of flavored pellets consumed following saline or naltrexone microinjection is shown for each 15 min postinjection. Closed symbols represent data from the naltrexone test sessions while open symbols represent data from the saline test sessions. (b) Cumulative consumption at the 90 min time point.

postinjection. This supports the idea that the selective enhancement of consumption of a preferred flavor by intra-NAcc DAMGO depends upon a subset of opioid sensitive NAcc neurons.

Histology

Histological analysis showed that the injector cannulae were successfully targeted to the NAcc (Fig. 6a, b).

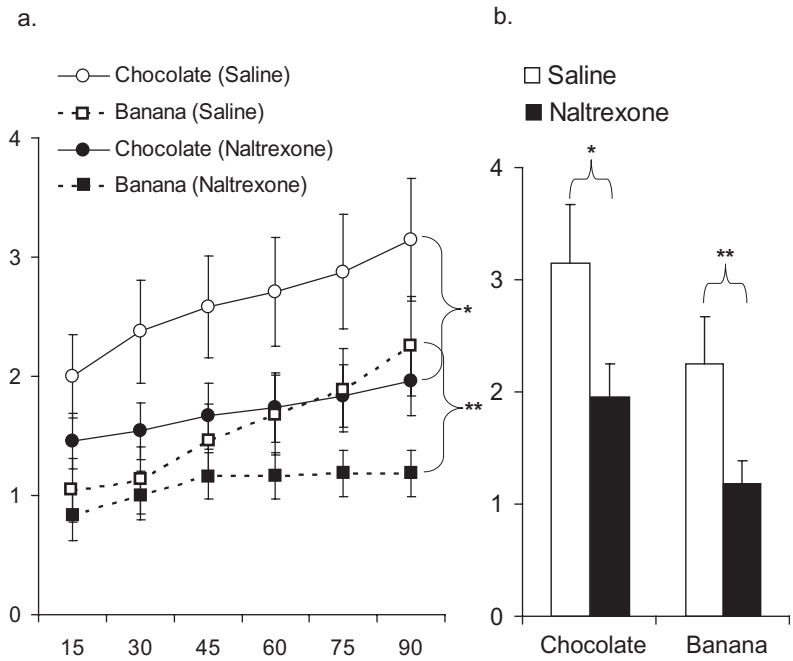

Fig. 4. Effect of systemic opioid receptor antagonist on consumption in a choice paradigm. (a) The number of flavored pellets consumed following saline or naltrexone s.c. injection is shown for each 15 min postinjection. Closed symbols represent data from naltrexone sessions while open symbols represent data from saline test sessions. (b) Cumulative consumption at the 90 min time point.

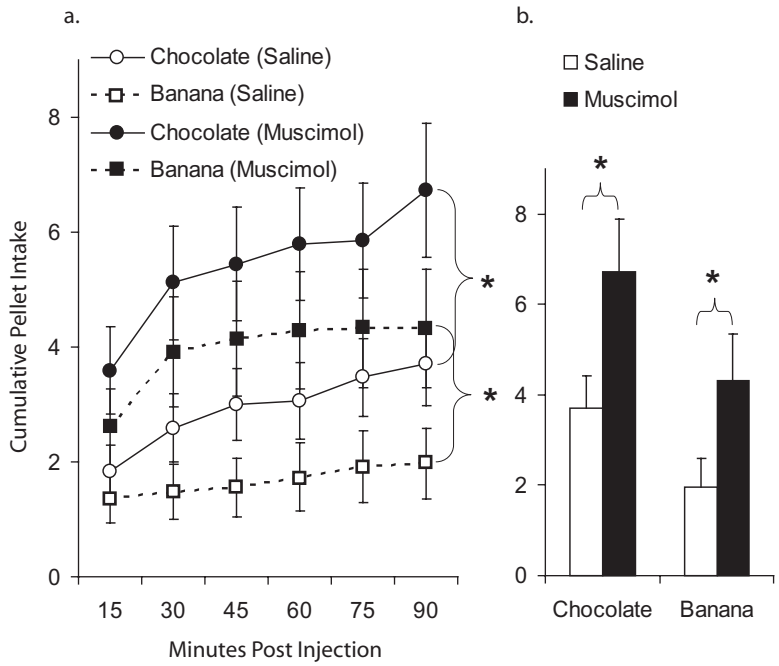

Fig. 5. Effect of intra-NAcc $GABA_A$ receptor agonist on consumption in a choice paradigm. (a) The number of flavored pellets consumed following saline or muscimol microinjection is shown for each 15 min postinjection. (b) Cumulative consumption at the 90 min time point.

DISCUSSION

The main purpose of this study was to isolate the contribution of taste to the enhancement of food intake by opioid agonists when macronutrient content is held constant. Although intra-NAcc DAMGO enhanced consumption of either flavored pellet to the same extent when they were presented separately, when presented at the same time in a choice paradigm, intra-NAcc DAMGO selectively increased, while intra-NAcc naltrexone selectively decreased, chocolate consumption. Since rats exhibit a relative preference for chocolate in the absence of opioid treatment, intra-NAcc MOP opioid administration enhances flavor-based food preference (i.e. palatability). As opposed to intra-NAcc administration, systemic naltrexone decreased consumption of both flavors equally. Thus, depending upon the situation, intra-NAcc DAMGO can either enhance intake of a range of palatable foods presented alone, or it can selectively enhance consumption of a preferred flavor when a choice is available. Furthermore, naltrexone injected into the NAcc, but not systemically, selectively decreases preference for the usually preferred flavor in a choice paradigm, indicating that endogenous opioids acting in the NAc contribute to flavor-based preference.

The effects of intra-NAcc DAMGO on consumption when the two types of flavored pellets were presented alone and when they were presented together are consistent with previous work. Intra-NAcc DAMGO significantly increases consumption of both bland chow and palatable sucrose when these are presented alone in non-deprived rats (Bakshi and Kelley, 1993a,b; Zhang and Kelley, 1997). When given a choice between foods, however, microinjection of DAMGO 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 or water unchanged (Kelley et al., 2002). Similarly, Kelley et al. (2002) have shown that intra-NAcc DAMGO enhances consumption of either a carbohydrate or fatty mash when they are presented alone (although fat consumption is increased significantly more), but only increases consumption of the fatty mash when both foods are presented simultaneously irrespective of the animals' baseline preferences (i.e. carbohydrate preferring rats still only increase their fat consumption after DAMGO microinjection) (Zhang et al., 1998). The present experiments confirm and extend those findings by showing that when given a choice between foods with identical macronutrient content (and texture) but different flavors, NAcc DAMGO selectively increases consumption of the food with the generally preferred taste. These two actions of MOP agonists in the NAcc are related but distinct. If food of only one flavor is present, MOP agonists will enhance feeding relative to other competing behaviors. On the other hand, if a flavor choice is presented, MOP agonists will selectively enhance intake of the generally preferred flavor.

The fact that intra-NAcc naltrexone selectively decreases chocolate consumption suggests a role for endogenous opioid release within the NAcc in flavor preference. Many studies have found that systemic and intracerebroventricularly administered naltrexone more effectively reduces consumption of palatable foods than bland chow (for review see (Bodnar 2004)). Furthermore, microinjection of

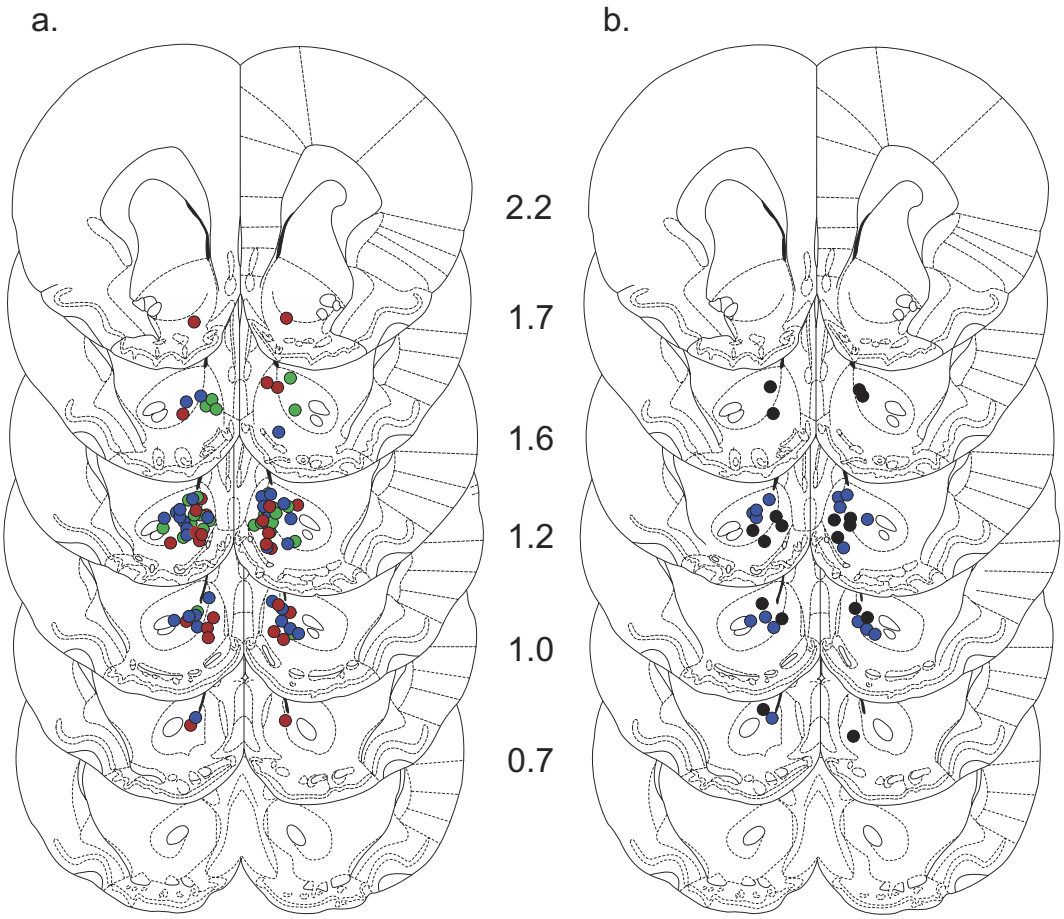

Fig. 6. Cannulae placements in the NAcc. (a) Green circles denote cannulae placements in rats microinjected with DAMGO and then given access to either chocolate or banana pellets. Red and blue circles denote cannulae placements in rats microinjected with DAMGO (red) or naltrexone (blue) and then given simultaneous access to pellets of both flavors. (b) Circles denote cannulae placements in rats microinjected with muscimol and then given simultaneous access to pellets of both flavors. Blue circles represent placements in animals that were previously used for naltrexone microinjections (they are identical to a subset of blue circles shown in a). Numbers denote millimeters anterior to bregma.

naltrexone into the NAcc decreases consumption of a sucrose solution in non-deprived rats but has no effect on chow consumption in food-deprived rats (Kelley et al., 1996). While no study to date has directly measured endogenous opioid release in the NAcc after consumption of a palatable food, the ability of naltrexone to reduce consumption suggests an important role for opioid signaling at this site in feeding. In the current study, the selectivity of naltrexone-induced decreases in consumption for the preferred flavor suggests an important role for endogenous opioids in flavor preference. The fact that naltrexone decreases consumption non-selectively when given systemically points to the importance of opioid receptors within the NAcc for palatability-related food preferences. It is possible that at higher concentrations, systemically administered naltrexone could have flavor specific effects. However, systemic naltrexone decreased consumption of both chocolate and banana pellets while intra-NAcc naltrexone had almost no effect on banana consumption. This difference further supports a role for NAcc endogenous opioids in flavor choice.

In the current study, different groups of rats were used in each experiment. This means that different rats were used to establish the flavor preference for chocolate than those that were used to investigate drugs effects. This raises the possibility that the rats in which we are investigating drug effects do not actually prefer chocolate to banana. This was done because there was substantial intra-trial variability in flavor preference (i.e. sometimes they eat more banana than chocolate) making it difficult to establish an individual rat's flavor preference. That being said, we believe that this is not a problem since when taken as a group, rats never consume more banana than chocolate pellets when given a choice between the two (e.g. Table 1, Figs. 2–5) (Woolley et al., submitted for publication) although this difference does not always reach significance in smaller sample sizes. Furthermore, no rats consistently chose banana over chocolate indicating that in general, chocolate is the preferred flavor.

Opioid signaling in different brain regions has different effects on feeding. For example, naltrexone selectively decreases consumption of a preferred diet when injected

into the central nucleus of the amygdala but nonspecifically reduces consumption after microinjection into the paraventricular nucleus of the hypothalamus (Glass et al., 2000). Because systemic morphine increases consumption based on animals' baseline preference (Gosnell et al., 1990), while intra-NAcc microinjection of the MOP agonist DAMGO increases consumption of fat irrespective of baseline preference (Zhang et al., 1998), opioid signaling has been proposed to be involved in either macronutrient choice or palatability depending upon the brain region (Levine and Billington, 2004). The current study, in conjunction with those of the Kelley group (Zhang et al., 1998), suggests that opioid signaling within a single neural structure (NAcc) can have both palatability and macronutrient specific effects.

To compare the effect on palatability-based choices of non-selective inhibition versus opioid microinjection, we microinjected muscimol into the NAcc and then gave rats a choice of flavored pellets. Muscimol increased consumption of both chocolate and banana pellets equally. This is distinctly different from the selective increase in chocolate consumption induced by intra-NAcc DAMGO. These results are consistent with previous findings that unlike MOP agonists, intra-NAcc muscimol increases consumption without altering the preference between fat and carbohydrate meals when presented together (Basso and Kelley, 1999). Finally, the flavor independent enhancement of food intake by muscimol in the NAcc is similar to the type of feeding induced by lateral hypothalamus stimulation (Stratford and Kelley, 1997; Reynolds and Berridge, 2001, 2002). Taken together, these previous results and the current findings highlight the importance of NAcc opioid actions to flavor preference.

CONCLUSION

In summary, the present study demonstrates that MOP receptor stimulation within the NAcc can have at least two separate effects: 1) to increase consumption of palatable foods in general and 2) to selectively increase consumption of a preferred flavor. This NAcc-mediated flavor preference cannot be accounted for by differences in macronutrient content because the pellets used in the present study are nutritionally identical. In addition, we found that intra-NAcc naltrexone selectively reduced consumption of a preferred flavor, thus implicating endogenous opioids in NAcc-mediated flavor preference. While a $GABA_A$ receptor agonist microinjected into the NAcc also increased feeding, this effect was independent of relative palatability; consumption of the more and less preferred flavors was increased equally. These differences highlight important functional differences between opioid and GABAergic signaling systems within the NAcc and support a specific role for intra-NAcc opioid signaling in flavor-based preference.

Acknowledgments—This work was supported by the state of California for medical research on alcohol and substance abuse through the University of California, San Francisco.

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(Accepted 27 June 2006)