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NUCLEUS ACCUMBENS OPIOID SIGNALING CONDITIONS SHORT-TERM FLAVOR PREFERENCES

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Abstract—Opioid signaling in the nucleus accumbens (NAcc) strongly modulates flavor-based food choice. To further investigate the role of opioid signaling in taste reward, we used a sensory specific satiety (SSS) paradigm to devalue specific flavors of nutritionally identical food pellets in rats. In the NAcc, infusion of a μ opioid (MOP) receptor selective agonist selectively increased consumption of a pre-fed flavor, thus reversing the SSS effect. Conversely, blockade of endogenous opioid signaling with the opioid antagonist naltrexone selectively decreased consumption of a recently consumed flavor, potentiating the SSS effect. No enhancement of consumption was observed if a delay of 3 h was imposed following the intra-NAcc MOP agonist indicating that there were no long-term changes in flavor preference. If a delay was introduced between the initial flavor exposure and the intra-NAcc MOP agonist infusion, pellet consumption was increased non-selectively (irrespective of flavor) suggesting that close temporal contiguity between flavor experience and NAcc opioid action is critical for the opioid effect on flavor preference. In contrast to opioid effects, inactivating NAcc neurons by local microinjection of muscimol (a $GABA_A$ agonist) increased consumption of both the pre-fed and non-pre-fed flavors equally. These results demonstrate that opioids released in the NAcc during consumption of palatable foods produce a selective and transient increase in preference for a recently sampled flavor.

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

When a choice of food items is available, the palatability of each food (i.e. its reward value as signaled by orosensory cues (Rolls, 2001)) is critical to deciding how much of each item will be consumed. Despite the critical importance to survival of such decisions, the neural mechanisms of palatability-based food reward and preference are poorly understood. One process that powerfully modulates palatability is sensory specific satiety (SSS), whereby the perceived pleasantness of a specific food and the preference for it decrease as more of it is consumed. For example, when people are pre-fed one particular food, they rate the pleasantness of its flavor lower relative to that of non-pre-fed foods (Rolls and Rolls, 1997).

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Abbreviations: DAMGO, D-Ala^2,N,Me-Phe^4,Gly-ol^5-enkephalin; MOP, μ opioid; NAcc, nucleus accumbens; SSS, sensory specific satiety.

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There is an extensive body of evidence implicating opioid signaling in food consumption, especially palatability-driven feeding (Kelley et al., 2002; Bodnar, 2004; Bodnar and Klein, 2004; Kelley, 2004). Opioid agonists increase, while antagonists decrease consumption of palatable foods much more than bland chow (Calcagnetti and Reid, 1983; Cooper, 1983; Berridge, 1996). Microinjection of D-Ala^2,N,Me-Phe^4,Gly-ol^5-enkephalin (DAMGO, a μ opioid (MOP) receptor selective agonist) into the nucleus accumbens (NAcc) preferentially increases consumption of palatable items (Zhang et al., 1998; Zhang and Kelley, 2002) as well as positive affective orofacial reactions to sweet tastes (Pecina and Berridge, 2005). Furthermore, opioid antagonists injected in the NAcc can significantly and preferentially reduce consumption of palatable foods, indicating that endogenous opioid release at this site contributes to assessment of palatability and food preference (Bodnar et al., 1995). These data implicate the NAcc in opioid-induced feeding and raise the possibility that this area is critical for opioid regulation of SSS.

The medial shell region of the NAcc exerts a powerful inhibitory effect on feeding. Blockade of glutamate receptors in this region increases, while activation of glutamate receptors decreases feeding (Maldonado-Irizarry et al., 1995; Stratford et al., 1998) (but see Echo et al., 2001). 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. Increasing levels of endogenous GABA in the NAcc by blocking GABA breakdown also increases feeding (Stratford and Kelley, 1997). These effects are specific to food; gnawing and water consumption are unaffected. However, 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).

We have previously investigated the roles of intra-NAcc MOP and $GABA_A$ receptor agonists in a flavor choice paradigm using two nutritionally identical but differently flavored palatable food pellets. When injected into the NAcc, DAMGO selectively increases, while naltrexone selectively decreases, consumption of the more preferred food (Woolley et al., 2006). In contrast, muscimol increases consumption of both flavors of pellet equally.

In the current study we investigated the roles of MOP and GABA-A receptor agonists in flavor preference under conditions promoting flavor specific satiety, i.e. using the SSS paradigm. Testing opioid effects in a SSS paradigm allows us to distinguish between possible mechanisms underlying opioid effects on preferred food intake. One

possibility is that opioids potentiate existing flavor preferences but do not themselves constitute a signal for palatability. If this is the case, then when given following pre-feeding with a particular flavor, DAMGO should, in a choice situation, selectively increase consumption of an alternative, non-pre-fed flavor because pre-feeding has devalued the first food and shifted preference to the alternative choice. This would increase the SSS effect. Alternatively, if 1) decreased endogenous opioid release following pre-feeding to satiety is itself a mechanism underlying the SSS effect, or 2) MOP receptor activation reinforces (i.e. increases the relative value) the flavor just consumed, MOP agonists should reverse the satiety effect (i.e. selectively increase consumption of the pre-fed food). Our results support the latter concept of MOP agonist function. Since microinjection of DAMGO throughout the NAcc increases consumption of palatable foods (Zhang and Kelley, 2000) we targeted our microinjections to the border of the shell and the core, as Ann Kelley's group has done previously (Zhang et al., 1998).

EXPERIMENTAL PROCEDURES

Animals

A total of 138 male rats (Long Evans, Charles River Laboratories, Wilmington, MA, USA) weighing between 270 and 480 g were used in the present studies. All procedures were approved by the University of California, San Francisco, Animal Care and Use Committee and conformed to international guidelines on the ethical use of animals. Every attempt was made to minimize the number of animals used and their suffering. 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. For control microinjections, coordinates for the target sites were 1.5 mm anterior, 1.1 mm lateral and 3.5 mm ventral from Bregma (i.e. dorsal striatum). Animals were allowed 4 days of recovery postsurgery.

Drugs and injections

For microinjections, DAMGO, a selective MOP agonist, naltrexone, a non-selective opioid antagonist, and muscimol, a selective $GABA_A$ receptor agonist, were obtained from Sigma Pharmaceuticals (St. Louis, MO, USA). All drugs were dissolved in 0.9% sterile saline (DAMGO: 0.25 μg per side, naltrexone: 20 μg per side, and muscimol: 50 ng per side). These doses were chosen because they are 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 total 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 (4 days), animals were extensively handled. In order to overcome taste neophobia, rats were brought into the testing room on four successive days and given 1 h 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 micro- nutrients (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 amount of effort encouraged the rats to consume all that they took and greatly facilitated consumption quantification because rats generally consumed each pellet entirely before extracting a new pellet. Every 15 min postinjection, the number of pellets remaining in the dispenser was counted and a visual inspection of the cage for dropped food was made. Rats were removed from their home cages for the duration of the microinjection and then immediately returned. Testing sessions were separated by at least 48 h. The SSS paradigm consisted of a pre-feeding phase that consisted of *ad libitum* access to pellets of one flavor for 1 h, an injection phase, and a postinjection test phase where rats were given simultaneous access to pellets of both flavors for 1.5 h. Before the pre-feeding phase, rats were in an *ad libitum*, non-deprived state. For the experiments that investigated time course effects, delays were introduced between the pre-feeding and injection stages as well as injection and postinjection test stages. During this 2 or 3 h delay, rats were provided water *ad libitum* but no food was available during this period. This mild deprivation had little effect on consumption since consumption was similar with and without this deprivation.

Data analysis

All data are expressed as mean$\pm$S.E.M. (standard error of the mean). Data were analyzed using repeated measures ANOVA with pharmacologic manipulation, pre-feeding and flavor as repeated measures. Post hoc comparisons were made using the Bonferroni correction.

Histology

After the completion of all testing, rats were anesthetized deeply with sodium pentobarbital 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 cut coronally into 45 μm sections, mounted and stained with Neutral Red. Sections were examined under the microscope in order to determine placement of micro-injector tips.

RESULTS

Intra NAcc DAMGO reverses SSS

Rats (n=16) were given 1 h *ad libitum* access to either banana or chocolate pellets. At the end of this hour, either DAMGO or saline was microinjected into the NAcc. Rats were then given 1.5 h of simultaneous *ad libitum* access to both chocolate- and banana-flavored pellets. All rats under-

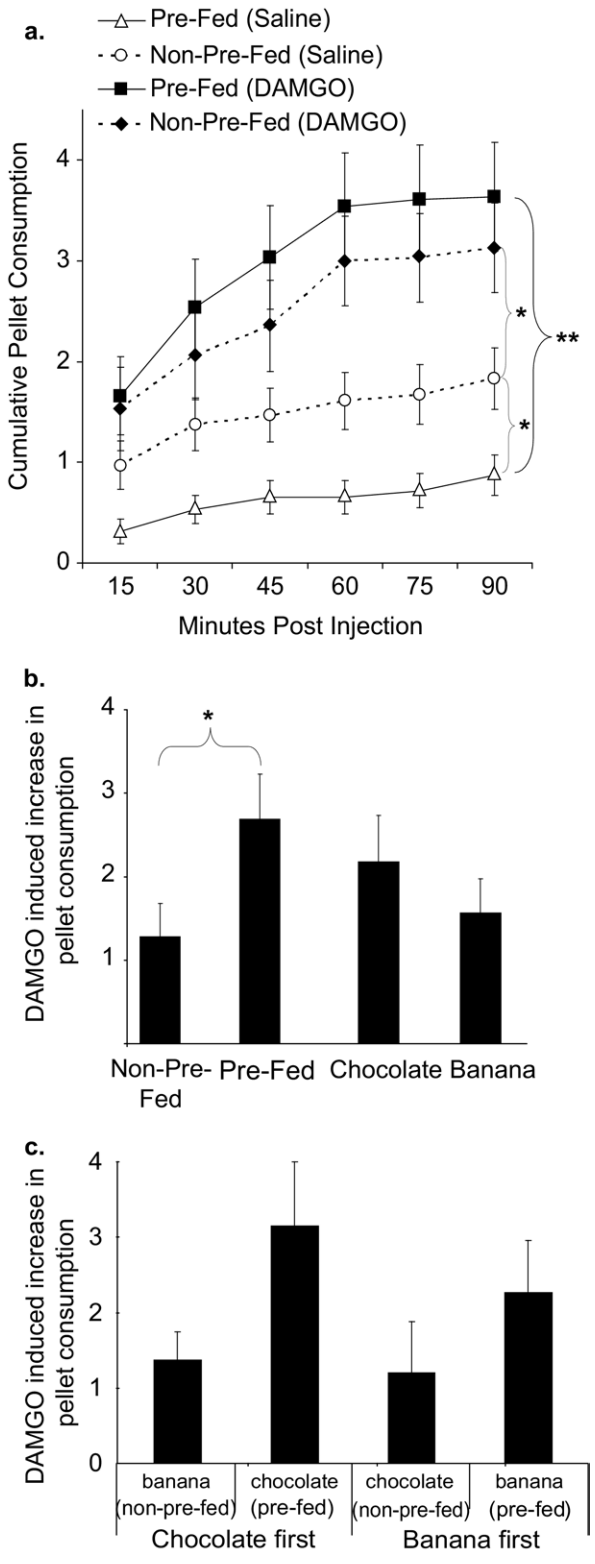


Fig. 1. Effects of intra-NAcc infusion of the MOP-specific agonist DAMGO on consumption in a SSS paradigm. "Pre-fed" represents the flavor which was pre-fed to the animal prior to microinjection. "Non-pre-fed" represents the flavor that was not pre-fed to the animal for all graphs. (a) The cumulative number of pellets consumed following saline or DAMGO microinjection is shown at 15 min intervals postinjection. Since both flavors were available after microinjection, filled symbols represent data from the saline test sessions while open symbols represent data from the drug test sessions for all graphs. Similarly, solid lines denote consumption of the pre-fed flavor while dashed lines denote consumption of the non-pre-fed flavor. (b) DAMGO-induced increases in consumption are shown. Bars indicate the total number of flavored pellets consumed following DAMGO minus the number consumed following saline microinjections at 90 min. Chocolate and banana consumption are averaged across different pre-fed and non-pre-fed conditions i.e. when chocolate is pre-fed and when banana is pre-fed. (c) Intra-NAcc opioid effects displayed by which flavor is pre-fed. Bars indicate number of flavored pellets consumed at 90 min following DAMGO minus the number consumed following saline microinjections. The two columns in the "Chocolate first" section come from trials where chocolate was the pre-fed food. The two columns in the "Banana first" section come from trials where banana was the pre-fed flavor. Labels in brackets indicate which flavor was pre-fed. (* $P<0.05$, ** $P<0.01$, † $P<0.1$ for all graphs.) DAMGO increases consumption of the pre-fed flavor significantly more than the non-pre-fed flavor, thereby reversing SSS.

went all four conditions and injection and flavor orders were randomized.

Whether chocolate or banana, rats receiving intra-NAcc saline ate significantly fewer pellets of the flavor that had been available during the pre-feeding period. When DAMGO was given in the NAcc following pre-feeding, this SSS effect was abolished and the rats consumed increased amounts of both the pre-fed and non-pre-fed flavored pellets (Fig. 1a). Strikingly, pre-fed food consumption in the test phase was increased much more than non-pre-fed food intake. Repeated measures ANOVA indicate that DAMGO significantly increased consumption [$F(1,15)=36.200$, $P<0.001$] and there was a significant drug×pre-feeding interaction [$F(1,15)=4.675$, $P<0.05$] (Fig. 1a). Post hoc mean contrasts performed on the 90 min time point indicated that there was a significant difference between consumption of the pre-fed and non-pre-fed foods only during saline administration. Together, this indicates that DAMGO increased consumption of the pre-fed food significantly more than the non-pre-fed food. This was confirmed by post hoc mean contrasts of the DAMGO induced increases in consumption ($P<0.05$) (Fig. 1b). While there were significant differences between consumption of chocolate and banana pellets with saline and with DAMGO administration [$F(1,15)=17.306$, $P<0.001$] (rats ate more chocolate pellets), there was no interaction between pharmacological manipulation and flavor (Fig. 1b). Further repeated measures ANOVAs indicate that the effect of DAMGO on consumption became significant by 15 min and the interaction between the effect of pre-feeding and pharmacologic manipulation became significant after 45 min of testing. The pattern of DAMGO-induced increases in consumption of the pre-fed and non-pre-fed foods was independent of flavor (Fig. 1c). The enhanced consumption of the pre-fed flavor is not due to a DAMGO-induced reduction in the rat's ability to perform taste discriminations since rats that have not been pre-fed maintain a clear preference for chocolate over banana following DAMGO administration (Woolley et al., 2006).

Anatomical specificity of the opioid effect

A previous mapping study found that DAMGO increased consumption when microinjected throughout the NAcc in a broad gradient, with ventral and lateral sites being the most effective (Zhang and Kelley, 2000). In those studies DAMGO in the dorsal striatum was also effective at increasing consumption, albeit at higher doses. To investigate the anatomical specificity of intra-NAcc DAMGO in the SSS paradigm, we microinjected DAMGO 2 mm dorsal to our previous target ($n=8$). Overall, this manipulation had minimal effects on consumption (i.e. no significant effect of pharmacologic manipulation or significant drug×pre-feeding or drug×flavor interactions). There was, however, a significant drug×pre-feeding×flavor interaction [$F(1,7)=11.293$, $P<0.05$]. This interaction was due to a significant effect of drug following pre-feeding with banana [$F(1,7)=11.055$, $P<0.05$] and a significant drug×pre-feeding interaction following pre-feeding with chocolate [$F(1,7)=5.858$, $P<0.05$] (these effects did not reach significance until the 90 min time point) (Fig. 2). Post hoc mean contrasts performed on the 90 min time point indicated that, in contrast to microinjections within the NAcc, dorsal injections produced a significant difference in consumption between saline and DAMGO conditions only for the non-pre-fed food ($P<0.05$). These effects are consistent with previous studies demonstrating that intraventricular and dorsal striatal DAMGO increases consumption of palatable foods. However, the fact that these effects are qualitatively different from those of intra-NAcc DAMGO injections supports the specificity of opioid receptors within the NAcc in modulating flavor preference under conditions of choice, i.e. preventing or reversing flavor specific satiety effects. They also rule out the possibility that the effects of our intra-NAcc opioid microinjections are due to backflow into the ventricles.

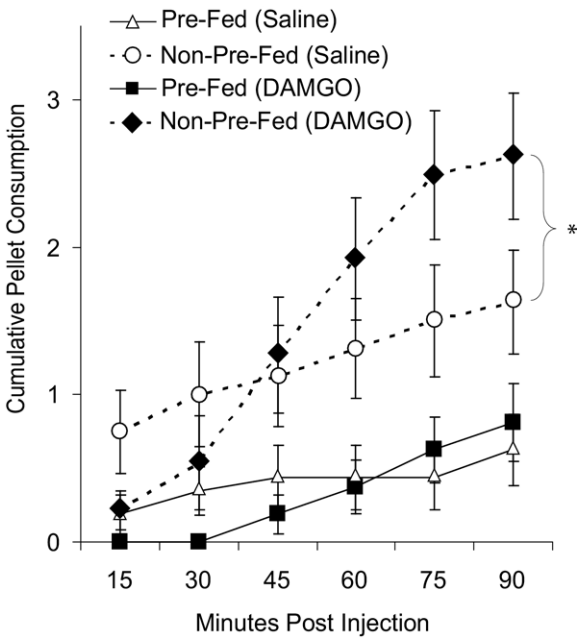


Fig. 2. Placement control. DAMGO was microinjected 2 mm dorsal to microinjections shown in Fig. 1. The cumulative number of pellets consumed following saline or DAMGO microinjection is shown for each 15 min postinjection. Note the delayed onset of the effect and that, in contrast to the effect in NAcc, at this dorsal site DAMGO did not enhance consumption of the pre-fed flavor.

Systemic morphine non-selectively increases consumption

To determine whether systemically delivered morphine (which has a relative preference for binding to the MOP receptor) has similar effects on preference to NAcc DAMGO, the SSS paradigm was repeated using s.c. morphine injections instead of NAcc DAMGO microinjections ($n=14$). In contrast to NAcc DAMGO, systemic morphine increased consumption of pellets of both flavors regardless of pre-feeding (repeated measures ANOVA at the 90 min time point indicated a significant drug effect [$F(1,13)=7.496$, $P<0.05$] but no drug×pre-feeding or drug×flavor interactions (Fig. 3a, b)). The significant effect of morphine on consumption emerged by 30 min.

Intra-NAcc naltrexone increases SSS

To test the contribution of endogenous opioids in the NAcc to the reward value of a flavor, we repeated the SSS paradigm with naltrexone microinfused into the NAcc ($n=22$). Surprisingly, naltrexone transiently *increased* consumption of the non-pre-fed food at 15 min after injection. Repeated measures ANOVA at the 15 min time point indicated that there was a significant drug×pre-feeding interaction [$F(1,18)=5.105$, $P<0.05$] and a trend for an overall effect of naltrexone on consumption [$F(1,18)=4.1322$, $P<0.1$] (Fig. 4a). Post hoc mean contrasts performed on the 15 min time point indicated that there was a significant difference in consumption between saline and naltrexone conditions only for the non-pre-fed food ($P<0.05$). These effects subsided at later time points. In contrast, toward the end of the testing session an inhibitory effect of naltrexone on consumption of the pre-fed food emerged. Repeated measures ANOVA at the 90 min time point indicated significant drug×pre-feeding [$F(1,18)=4.713$, $P<0.05$] (Fig. 4a) and a trend for drug×pre-feeding×flavor [$F(1,18)=4.373$, $P<0.1$] interactions. Post hoc mean contrasts performed on the 90 min time point indicated that there is a significant naltrexone induced decrease in consumption only for the pre-fed food ($P<0.05$). This effect was primarily accounted for by a significant naltrexone-induced decrease in consumption of chocolate when it was the pre-fed food (Fig. 4b) ($P<0.01$). Given the small quantities consumed when banana is the pre-fed food (saline-treated animals consumed an average of 0.32 (±0.13) pellets while naltrexone-treated animals consumed 0.21 (±0.10) banana pellets in 90 min), this flavor specific difference is probably due to a floor effect when banana is the pre-fed item.

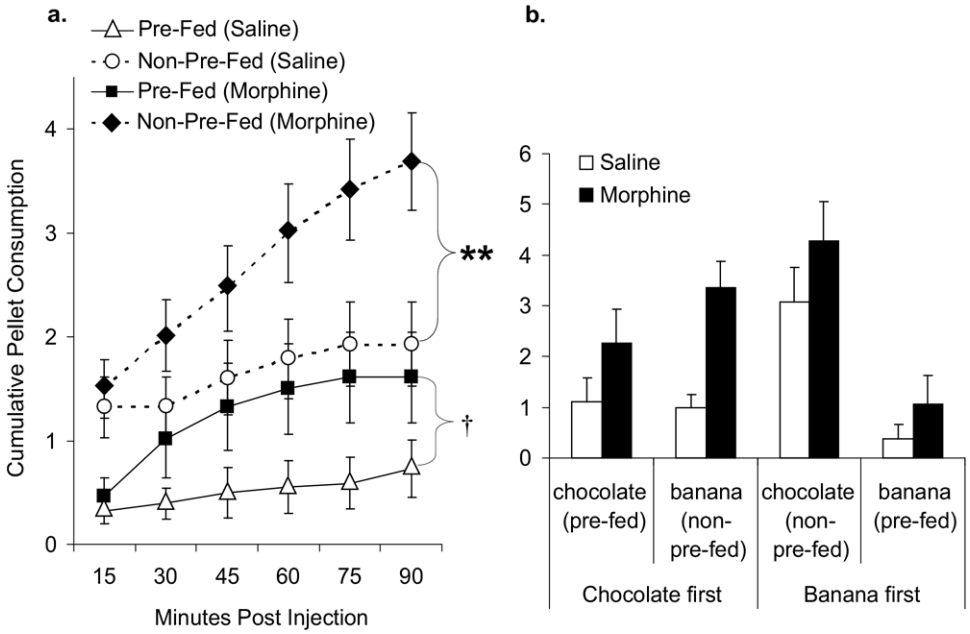


Fig. 3. Effect of *systemic* morphine on consumption in a SSS paradigm. (a) The cumulative number of pellets consumed following saline or morphine s.c. injection is shown at 15 intervals postinjection. Systemic morphine preferentially enhances consumption of the non-pre-fed flavor. (b) Systemic morphine effects displayed by which flavor is pre-fed. Bars indicate cumulative number of flavored pellets consumed at 90 min following saline or morphine s.c. injection. The two columns in the "Chocolate first" section come from trials where chocolate was the pre-fed food. The two columns in the "Banana first" section come from trials where banana was the pre-fed flavor. Labels in brackets indicate which flavor was pre-fed.

Systemic naltrexone reduces consumption independent of the pre-fed flavor

We used s.c. injections of naltrexone ($n=24$) to determine whether it has similar effects systemically as it has in the NAcc. In contrast to intra-NAcc naltrexone, systemically administered naltrexone decreased consumption equally for the pre-fed and non-pre-fed foods. Systemically administered naltrexone induced a preferential and late emerg-

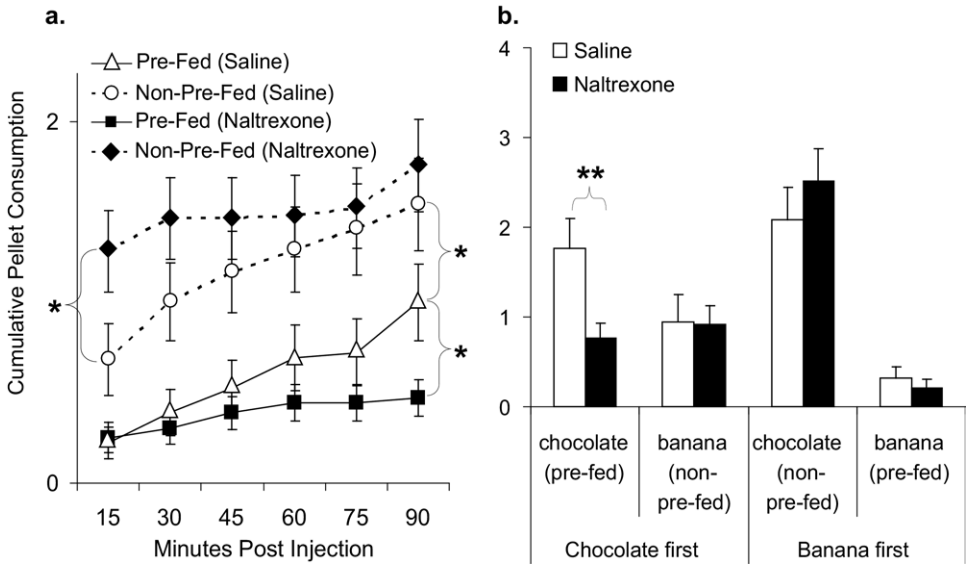


Fig. 4. Effects of intra-NAcc opioid antagonism on consumption in a SSS paradigm. (a) The cumulative number of pellets consumed following saline or naltrexone microinjection is shown at 15 min intervals postinjection. Naltrexone in the NAcc selectively decreases consumption of the pre-fed flavor thereby increasing SSS. (b) Intra-NAcc naltrexone effects displayed by which flavor is pre-fed. Bars indicate cumulative number of flavored pellets consumed at 90 min following saline or naltrexone microinjections. The two columns in the "Chocolate first" section come from trials where chocolate was the pre-fed food. The two columns in the "Banana first" section come from trials where banana was the pre-fed flavor. Labels in brackets indicate which flavor was pre-fed. While more chocolate than banana pellets were consumed both when chocolate was the pre-fed and the non-pre-fed flavor, the difference was smaller when chocolate was the pre-fed flavor consistent with SSS.

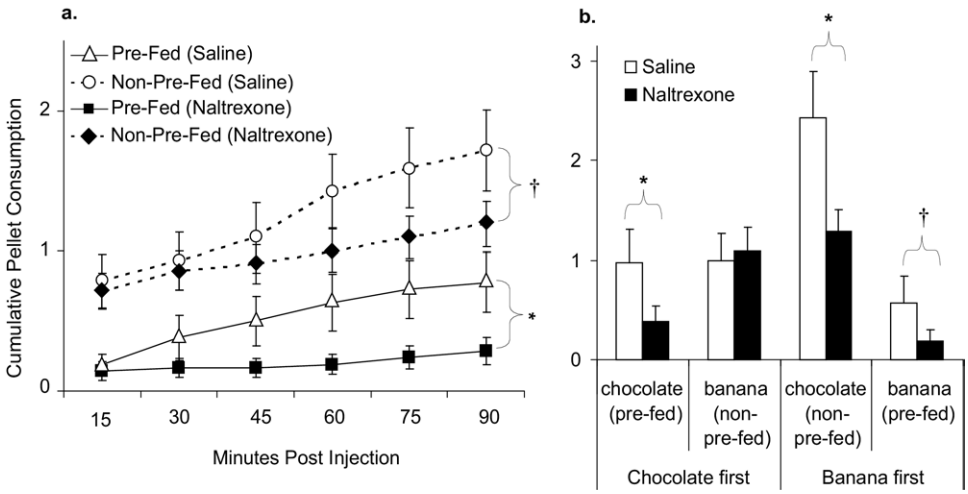


Fig. 5. Effects of systemic opioid antagonism on consumption in a SSS paradigm. (a) The cumulative number of pellets consumed following saline or naltrexone s.c. injection is shown at 15 min intervals postinjection. Systemic naltrexone reduces the consumption of both pre-fed and non-pre-fed flavors. (b) Systemic naltrexone effects displayed by which flavor is pre-fed. Bars indicate cumulative number of flavored pellets consumed at 90 min following saline or naltrexone s.c. injection. The two columns in the "Chocolate first" section come from trials where chocolate was the pre-fed food. The two columns in the "Banana first" section come from trials where banana was the pre-fed flavor. Labels in brackets indicate which flavor was pre-fed.

ing reduction in chocolate intake when rats were pre-fed chocolate. In contrast to intra-NAcc naltrexone, however, when given systemically, it also reduced chocolate intake when banana was the pre-fed flavor. Repeated measures ANOVA indicated that s.c. naltrexone significantly decreased consumption [$F(1,23)=8.050$, $P<0.01$] (Fig. 5a). There was a trend for a drug×flavor interaction [$F(1,23)=3.128$, $P<0.1$] but no significant drug×pre-feeding interaction. This trend was due to a larger effect of naltrexone on chocolate consumption (Fig. 5b). The significant effect of systemic naltrexone on consumption emerged by 60 min and the trend for an interaction between naltrexone and flavor emerged by 75 min.

The time course of the opioid effect

Using the same SSS paradigm, we introduced a 2 h delay between pre-feeding and DAMGO microinjection. Rats ($n=21$) were given 1 h *ad libitum* access to either banana or chocolate pellets. At the end of this hour, the pellet dispenser was removed from the cage and the rats remained in their home cage with *ad libitum* access to water but not food for 2 h. Following this delay, animals were microinjected with either DAMGO or saline and then immediately given 1.5 h simultaneous *ad libitum* access to both chocolate- and banana-flavored pellets. All rats underwent all four conditions and injection and flavor orders were randomized.

After a 2 h delay, intra-NAcc DAMGO non-selectively increased consumption of both flavors (repeated measures ANOVA at the 90 min time point revealed significant effects of drug [$F(1,20)=44.351$, $P<0.001$] and pre-feeding [$F(1,20)=8.06$, $P<0.05$] but no drug×pre-feeding or drug×flavor interactions (Fig. 6a, b)). These results indicate that the flavor specificity of the MOP-agonist effect is lost if a delay is introduced between flavor sampling and intra-NAcc DAMGO infusion.

To further examine the time course of DAMGO's conditioning effects in the NAcc, the same SSS paradigm was used but a 3 h delay was interposed between microinjection and simultaneous access to both types of pellets. Rats ($n=16$) were given 1 h *ad libitum* access to either banana or chocolate pellets. At the end of this hour, the pellet dispenser was removed from the cage and the rats remained in their home cage with *ad libitum* access to water but not food. Immediately following pre-feeding, animals were microinjected with either DAMGO or saline. Three hours later, rats were given 1.5 h simultaneous *ad libitum* access to both chocolate- and banana-flavored pellets. All rats underwent all four conditions and injection and flavor orders were randomized. Repeated measures ANOVA showed no significant effects of DAMGO after this delay period (Fig. 6c) including no significant drug×flavor or drug×pre-feeding interactions (Fig. 6d) although there was a significant effect of pre-feeding [$F(1,15)=15.372$, $P<0.005$]; even after a 3 h delay animals still consumed more of the non-pre-fed food.

Intra-NAcc muscimol has no effect on SSS

Finally, because GABAergic signaling in the NAcc has been shown to modulate food intake, we tested the effects of intra-NAcc muscimol injection in the SSS paradigm. Rats ($n=17$) were given 1 h *ad libitum* access to either banana or chocolate pellets. At the end of this hour, rats were microinjected with either muscimol or saline. Rats were then given 1.5 h simultaneous *ad libitum* access to both chocolate- and banana-flavored pellets. All rats underwent all four conditions and injection and flavor orders were randomized.

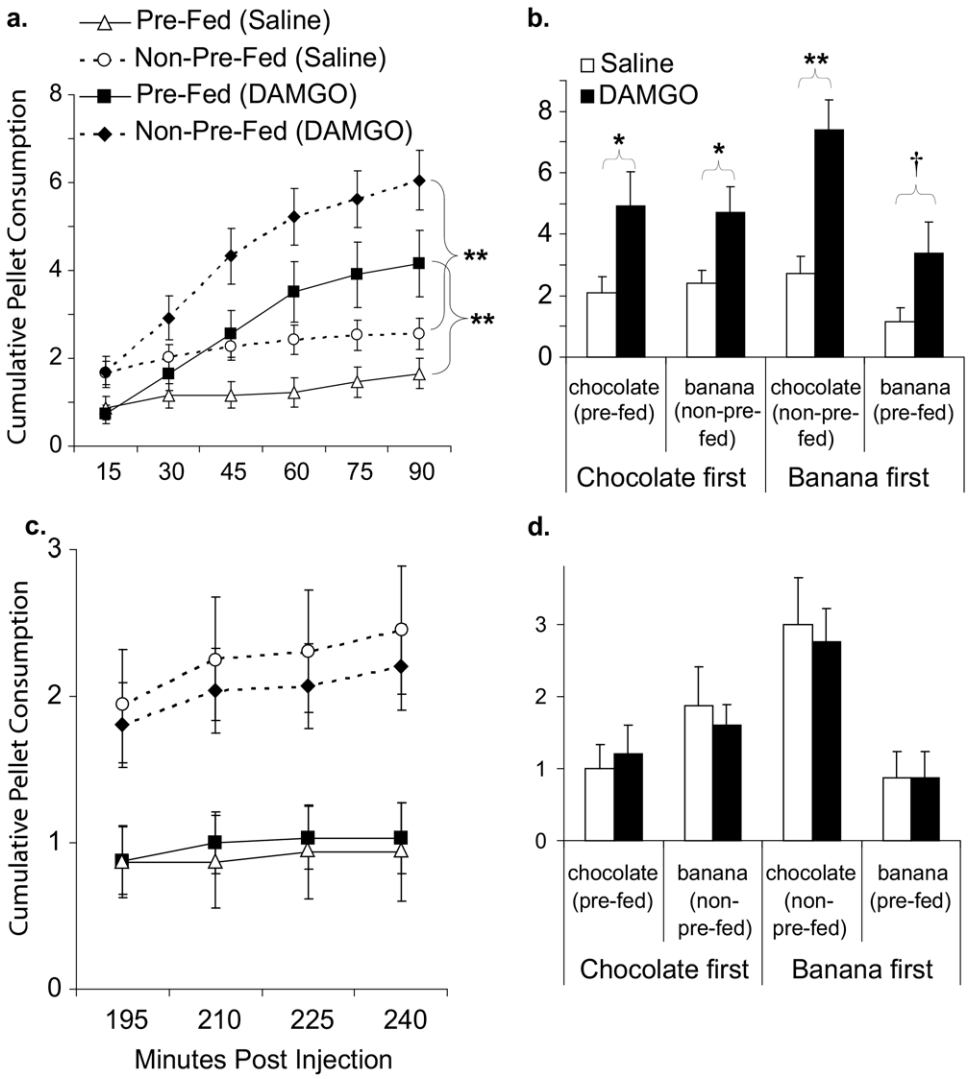
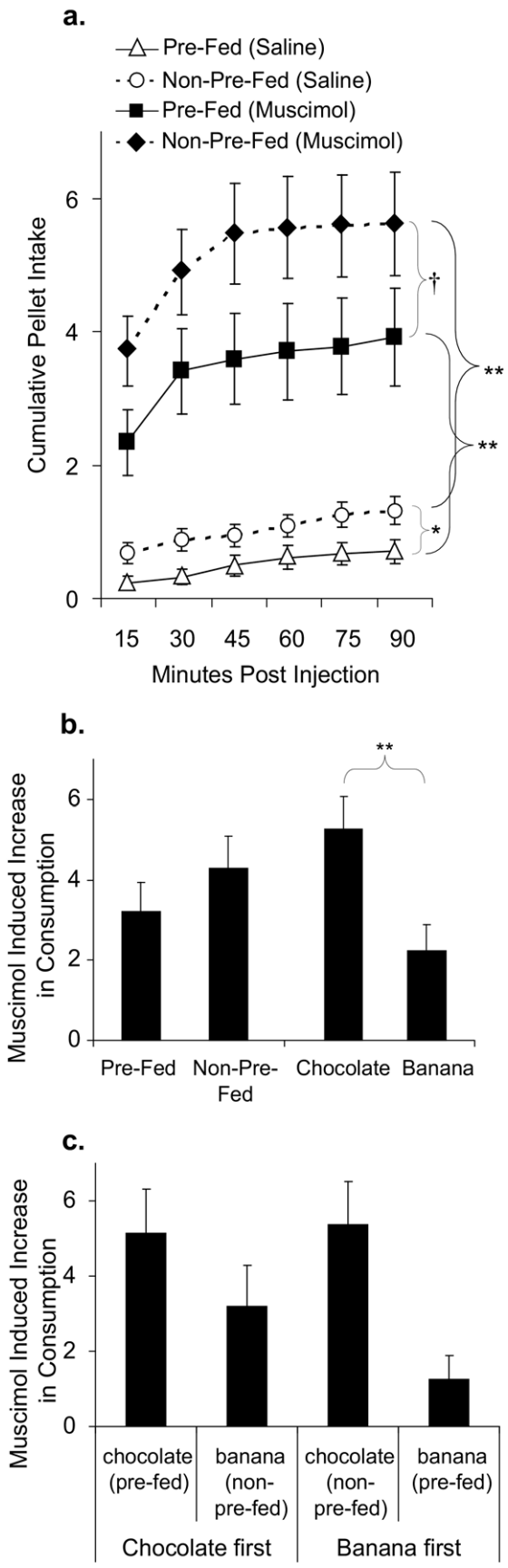


Fig. 6. Effects of an intra-NAcc MOP agonist on consumption in a SSS paradigm when (a, b) a 120 min delay between pre-feeding and microinjection or (c, d) a 180 min delay was introduced between microinjection and pellet access. (a, c) The cumulative number of pellets consumed following saline or DAMGO microinjection is shown for each 15 min after flavored pellets were introduced to the cage. (b, d) Intra-NAcc DAMGO effects displayed by which flavor is pre-fed. Bars indicate cumulative number of flavored pellets consumed at (b) 90 or (d) 240 min (180 min delay plus 60 min of pellet access) following saline or DAMGO microinjection. The columns in the "Chocolate first" section come from trials where chocolate was the pre-fed food. The columns in the "Banana first" section come from trials where banana was the pre-fed flavor. Labels in brackets indicate which flavor was pre-fed. When a delay is introduced between pre-feeding and microinjection, consumption is increased non-selectively. When a delay is introduced between microinjection and simultaneous pellet access, DAMGO no longer increased consumption.

Unlike DAMGO, intra-NAcc muscimol increased consumption of both flavors non-selectively, regardless of which flavor was pre-fed. Repeated measures ANOVA indicated that muscimol significantly increased consumption [$F(1,16)=61.662$, $P<0.001$] and there was no significant drug×pre-feeding interaction (Fig. 7a). There was a significant drug×flavor interaction [$F(1,16)=6.995$, $P<0.05$] due to a significantly larger effect of muscimol on chocolate consumption (Fig. 7b, c). Further repeated measures ANOVAs indicate that the significant effect of muscimol on consumption and the significant interaction between flavor and pharmacologic manipulation both emerged by 15 min postinjection. These results suggest that while NAcc MOP receptor activation promotes consumption of palatable foods when no choice is given and enhances preference for a recently consumed food when choice is available, $GABA_A$ receptor activation at the same sites non-selectively increases consumption without changing relative flavor preference.

Histology

Histological analysis showed that the injector cannulae were successfully targeted to the NAcc (Fig. 8a, c, d) and that the injector cannulae for the anatomic controls were placed in the dorsal striatum, lateral septum and ventricle (Fig. 8b).



DISCUSSION

We used the SSS paradigm to devalue a specific flavor by pre-feeding with that flavor and then allowing rodents to choose between that flavor and an alternative non-pre-fed flavor. Typically, rodents will preferentially consume a flavored food that is different from the one just consumed. We used this method to investigate how exogenous and endogenous opioids contribute to the reward value of food as signaled by orosensory cues. Infusion of the MOP-receptor selective agonist DAMGO into the NAcc reversed SSS and preferentially increased consumption of the pre-fed food. Conversely, naltrexone in the NAcc, which presumably blocks endogenous opioid signaling, increased SSS; i.e. it selectively decreased consumption of the pre-fed food. Neither systemic naltrexone, systemic morphine nor muscimol inactivation of the NAcc reproduced these effects, thus the flavor specific regulation of preference and consumption appears to be a function of opioids in the NAcc. If a delay is introduced between pre-feeding and microinjection, DAMGO no longer reverses SSS indicating that close temporal pairing of the opioid action with food consumption is critical for the flavor specificity of the MOP agonist effect. Finally, intra-NAcc DAMGO does not induce long term changes in flavor preference; after a 3-h delay, DAMGO-induced changes have receded completely. Taken together, these results demonstrate that consumption of a palatable food combined with the release of an endogenous MOP agonist within the NAcc conditions short term flavor preferences.

The observation that consumption of the pre-fed, devalued flavor is increased more than the non-pre-fed, and therefore non-devalued flavor argues that DAMGO increases consumption based on the recently experienced food regardless of its intrinsic flavor. Our results are consistent with the hypothesis that MOP opioid agonists acting in the NAcc cause a short term increase in preference for a recently consumed food.

In order to explore the anatomical specificity of the DAMGO effect on consumption, we repeated the SSS paradigm with DAMGO microinjections 2 cm dorsally to our intra-NAcc microinjections. First, DAMGO microinjections outside the NAcc did not reproduce the intra-NAcc's effects. Furthermore, we found that, unlike intra-NAcc

Fig. 7. Effects of an intra-NAcc $GABA_A$ receptor agonist on consumption in a SSS paradigm. (a) The cumulative number of pellets consumed following saline or muscimol microinjection is shown at 15 min intervals postinjection. Muscimol induced significant but non-selective increases in consumption of both pre-fed and non pre-fed flavors. (b) Bars indicate the total number of flavored pellets consumed following muscimol minus the number consumed following saline micro-injections at 90 min. Chocolate and banana consumption are averaged across different pre-fed and non-pre-fed conditions i.e. when chocolate is pre-fed and when banana is pre-fed. (c) Intra-NAcc muscimol effects displayed by which flavor is pre-fed. Bars indicate number of flavored pellets consumed at 90 min following muscimol minus the number consumed following saline micro-injections. The two columns in the "Chocolate first" section come from trials where chocolate was the pre-fed food. The two columns in the "Banana first" section come from trials where banana was the pre-fed flavor. Labels in brackets indicate which flavor was pre-fed.

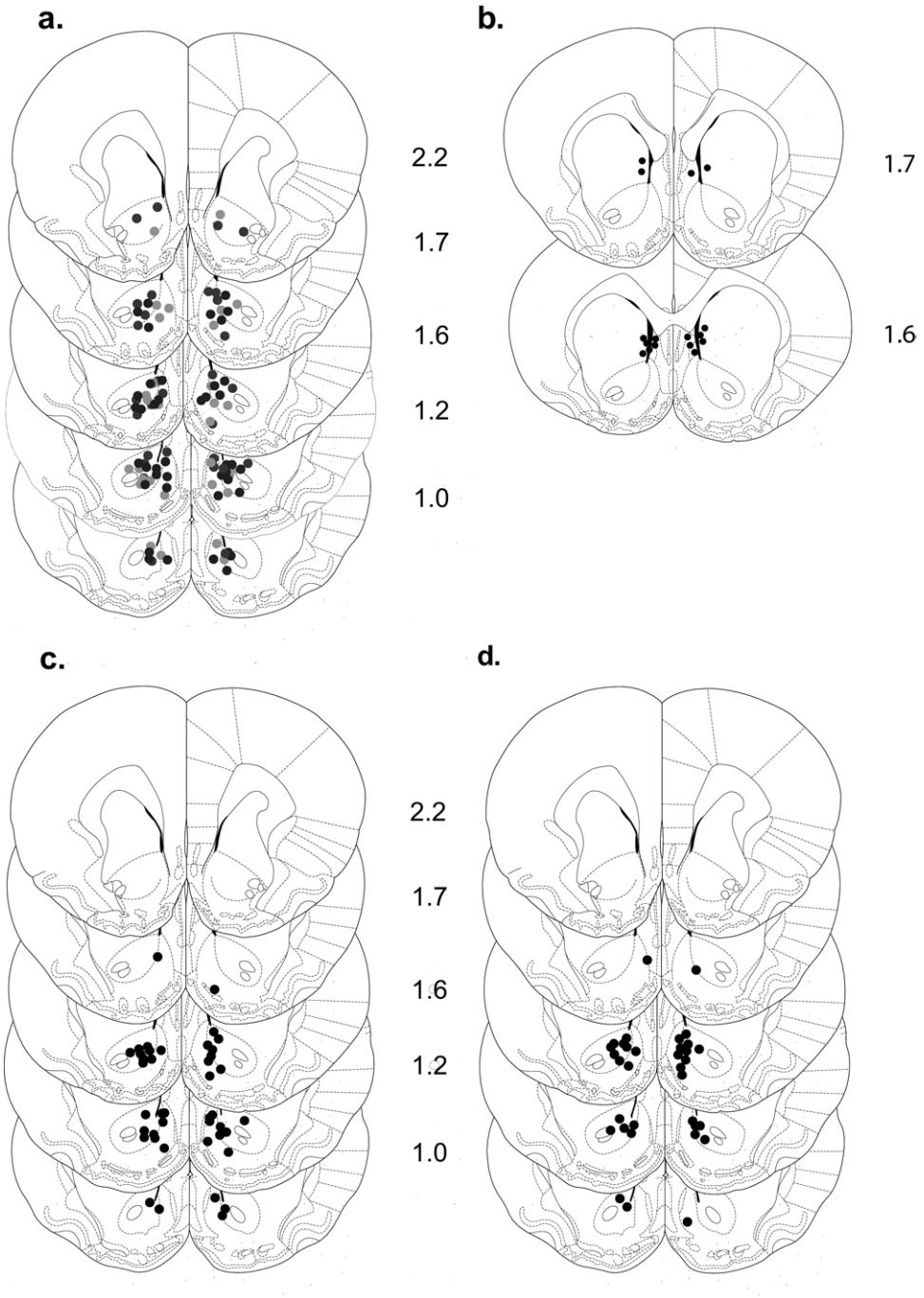


Fig. 8. Cannulae placements in the NAcc. (a) Red circles denote cannulae placements in rats microinjected with DAMGO in a SSS paradigm. Green circles denote cannulae placements in rats microinjected with DAMGO in the same SSS paradigm but with a 3 h delay interposed between microinjection and simultaneous access to both types of pellets. Blue circles denote cannulae placements in rats microinjected with DAMGO in the same SSS paradigm but with a 2 h delay interposed between pre-feeding and microinjection. (b) Black circles denote cannulae placements in rats microinjected with DAMGO 2 mm dorsal to our previous microinjections. Cannulae placements in the NAcc. (c) Black circles denote cannulae placements in rats microinjected with naltrexone in a SSS paradigm. (d) Black circles denote cannulae placements in rats microinjected with muscimol in a SSS paradigm. Numbers denote millimeters anterior to bregma.

DAMGO, systemic morphine increased consumption non-specifically, i.e. regardless of which flavor was pre-fed. This difference could be due to morphine's effects either at opioid receptors other than the MOP or at sites other than the NAcc, in the brain or periphery. Taken together, these results highlight the specificity of both the MOP receptor and the NAcc on SSS. The present data indicate that NAcc MOP agonists modulate flavor preference in a choice par-

adigm. The effects of intra-NAcc naltrexone microinjection in the SSS paradigm favor the idea that MOP agonists in NAcc act to enhance the palatability of a food that has just been eaten. If opioid signaling in the NAcc transiently reinforces consumption of the just-sampled food, naltrexone should, as observed, selectively decrease consumption of the pre-fed flavor. Surprisingly, we also found that naltrexone transiently increased consumption of the non-pre-fed food. Since naltrexone is a non-selective opioid antagonist, this effect may be due to its actions at opioid receptors other than the MOP.

Systemic MOP agonists can condition odor and flavor preferences (Kehoe and Blass, 1986; Lynch, 1986; Shide and Blass, 1991). Studies from the Bodnar laboratory, however, argue against a role of endogenous opioids in flavor preference conditioning (Yu et al., 1999; Baker et al., 2004). In order to investigate these discrepancies, we repeated the SSS paradigm but instead of microinjecting naltrexone into the NAcc we administered naltrexone systemically. We found that, unlike intra-NAcc naltrexone, systemic naltrexone decreases consumption independent of recent orosensory experience (i.e. the flavor that was pre-fed). This difference between systemic and NAcc naltrexone must depend on antagonism of opioid receptors outside the NAcc and illustrates the specificity of NAcc opioid receptors in flavor preference conditioning.

Unlike DAMGO, muscimol injection in the NAcc increased consumption for both the pre-fed and non-pre-fed foods. This is consistent with previous findings that NAcc muscimol effects on feeding are relatively independent of palatability (Basso and Kelley, 1999; Zhang et al., 2003). Differences in receptor distribution patterns, local microcircuit anatomy or cellular physiological effects must account for these differences between DAMGO and muscimol. One possible explanation for the difference is that in the NAcc, GABA agonists directly and non-selectively inhibit all medium spiny neurons by a postsynaptic mechanism, whereas opioid peptides presynaptically inhibit GABAergic release (Yuan et al., 1992). Thus opioids have the potential to selectively control specific inputs, perhaps those that relay taste information. This difference may contribute to the divergent effects of the two drugs. Alternatively, the different drug effects may be due to actions on different subsets of NAcc neuron. In electrophysiological studies, two distinct functional classes of NAcc neurons relating to consummatory behavior have been described (Taha and Fields, 2005). One group of neurons shows inhibitions prior to and during licking bouts that are insensitive to the palatability of the consumed liquid (sucrose). Increased activity in these neurons appears to have a general inhibitory effect on consummatory behavior, independent of palatability. By inhibiting these neurons, muscimol could non-specifically enhance consummatory behavior. A second class of neurons showed excitations that encoded the relative palatability of or preference for sweeter sucrose solutions. DAMGO in the NAcc might increase consumption of a preferred food item by selectively disinhibiting these palatability/preference encoding NAcc neurons.

In order to investigate the time course of opioid-induced taste conditioning we microinjected DAMGO into the NAcc in a SSS paradigm with introduced delays. We found that interposing a delay between pre-feeding and microinjection abolished the specificity of the DAMGO effect for the pre-fed item; it increased consumption non-specifically. In contrast, if a delay is interposed between microinjection and pellet access, DAMGO has no effects on consumption. These results indicate that intra-NAcc DAMGO preferentially enhances the reward value of recently sampled flavors and that this enhancement does not produce long term changes in flavor preference.

Substantial evidence implicates opioid signaling specifically in maintenance of ongoing feeding bouts (i.e. regulation of meal duration as opposed to meal initiation) (Kirkham and Blundell, 1984, 1986; McLaughlin and Baile, 1984; Kirkham and Cooper, 1988a,b; Rudski et al., 1994a,b; Glass et al., 1999). For example, systemic naltrexone decreases meal length and extends the post-meal interval but does not affect meal frequency or feeding rate in freely-feeding rats (Kirkham and Blundell, 1987). Furthermore, extensive evidence indicates that opioid signaling plays a more substantial role in non-homeostatic (palatability) than in calorie deprivation-induced feeding (Sanger and McCarthy, 1980; Levine et al., 1995; Cleary et al., 1996). Thus, systemic naltrexone more effectively decreases consumption in non-deprived than in deprived animals (Giraudo et al., 1993). In one recent study, naloxone (a non-selective opioid antagonist drug similar to naltrexone) dose-dependently reduced consumption of palatable food in sated rats but had no effect in food-restricted rats (Barbano and Cador, 2006). The sensitivity of opioid signaling to deprivation status and food palatability, coupled with the finding that opioid antagonists specifically shorten feeding bouts, indicates that endogenous opioid release specifically and transiently promotes palatability-driven feeding in excess of current metabolic demands. Such a mechanism may function to enhance consumption of rarely encountered but energy dense foods. The current finding that opioid signaling within the NAcc produces short term changes in flavor preference is consistent with this hypothesis. We hypothesize that sensory features of palatable foods cause opioid release which in turn promotes continued consumption of these foods beyond current homeostatic energy needs. When DAMGO is microinjected into the NAcc following pre-feeding, this artificial stimulation mimics natural opioid release, counteracts SSS and promotes over-consumption of the food just eaten compared with the non-pre-fed food. When DAMGO levels fall, however, this increased preference subsides.

MOP agonists in the NAcc increase, while antagonists decrease, consumption of palatable foods. Furthermore, endogenous opioids are released following consumption of palatable items. The question concerning opioid signaling that remains unanswered is exactly which aspects of the taste experience and consumption behavior are modulated by opioid stimulation. If the magnitude of endogenous opioid release following palatable food consumption only signals the level of its rewarding or positive motivational

properties (as supported by antagonist studies), why do exogenous agonists selectively increase consumption of already palatable items and not all foods equally or even less palatable foods more than palatable foods? In other words, why does exogenous opioid infusion fail to 'wash out' differences in opioid release (and thereby preference) for foods of different palatability? One possibility is that opioid release both signals the reward value of a sampled taste and promotes consumption of recently sampled tastes. These two distinct actions could explain why opioid antagonists prevent both the development and expression of taste preferences (since there is more opioid release to preferred tastes) while agonists can reverse satiety effects and enhance already established preferences (since they powerfully condition a preference for recently sampled tastes). The current demonstration that intra-NAcc MOP receptor stimulation modulates flavor preferences in a choice paradigm extends previous studies by providing evidence for this double action hypothesis.

CONCLUSION

In conclusion, the present findings, in concert with our previous results, demonstrate that intra-NAcc DAMGO has at least three effects on consumption: 1) it increases consumption of palatable foods, 2) it selectively increases consumption of a more preferred flavor, and 3) when a choice of flavors is available, it preferentially increases consumption of a recently sampled palatable food and reverses the satiety effect of pre-feeding. Specifically, MOP agonists transiently increase, while non-selective opioid antagonists decrease, consumption of recently consumed foods. These effects are not replicated by systemic opioid manipulations or non-selective NAcc inactivation. These results highlight the central role of NAcc opioid receptors in flavor-based decisions about food consumption.

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