Behavioral/Systems/Cognitive

Induction of Hyperphagia and Carbohydrate Intake by μ-Opioid Receptor Stimulation in Circumscribed Regions of Frontal Cortex

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Frontal cortical regions are activated by food-associated stimuli, and this activation appears to be dysregulated in individuals with eating disorders. Nevertheless, frontal control of basic unconditioned feeding responses remains poorly understood. Here we show that hyperphagia can be driven by μ-opioid receptor stimulation in restricted regions of ventral medial prefrontal cortex (vmPFC) and orbitofrontal cortex. In both *ad libitum*-fed and food-restricted male Sprague Dawley rats, bilateral infusions of the μ-opioid agonist [D-Ala2, N-Me-Phe4, Gly5-ol]-enkephalin (DAMGO) markedly increased intake of standard rat chow. When given a choice between palatable fat-enriched versus carbohydrate-enriched test diets, intra-vmPFC DAMGO infusions selectively increased carbohydrate intake, even in rats with a baseline fat preference. Rats also exhibited motor hyperactivity characterized by rapid switching between brief bouts of investigatory and ingestive behaviors. Intra-vmPFC DAMGO affected neither water intake nor nonspecific oral behavior. Similar DAMGO infusions into neighboring areas of lateral orbital or anterior motor cortex had minimal effects on feeding. Neither stimulation of vmPFC-localized δ-opioid, κ-opioid, dopaminergic, serotonergic, or noradrenergic receptors, nor antagonism of D1, 5HT1A, or α- or β-adrenoceptors, reproduced the profile of DAMGO effects. Muscimol-mediated inactivation of the vmPFC, and intra-vmPFC stimulation of κ-opioid receptors or blockade of 5-HT2A (5-hydroxytryptamine receptor 2A) receptors, suppressed motor activity and increased feeding bout duration—a profile opposite to that seen with DAMGO. Hence, μ-opioid-induced hyperphagia and carbohydrate intake can be elicited with remarkable pharmacological and behavioral specificity from discrete subterritories of the frontal cortex. These findings may have implications for understanding affect-driven feeding and loss of restraint in eating disorders.

Introduction

There is much current interest in investigating how specific neurotransmitters and peptides act within limbic corticostriatal circuits to drive basic food intake, with the goal of understanding the neural control of "nonhomeostatic" feeding. Previous work has demonstrated that opioid peptides are remarkably effective at driving food intake and/or enhancing taste hedonics from specific subcortical sites, notably the nucleus accumbens (Acb), the central nucleus of the amygdala, and ventral pallidum (Gosnell, 1988; Glass et al., 1999; Baldo and Kelley, 2007; Smith and Berridge, 2007). One commonality among these sites is strong connectivity with diencephalic feeding systems. Indeed, recent theories suggest that these sites represent discrete nodes of a forebrain network that exerts descending control over hypothalamic feeding circuits (Stratford et al., 1999; Swanson, 2000; Berthoud, 2004; Kelley et al., 2005; Petrovich et al., 2005).

Another structure well placed to participate in this network is the prefrontal cortex (PFC). Several subregions of the PFC, including the infralimbic, prelimbic, and ventral orbital regions, send strong projections to feeding-related nodes such as the lateral hypothalamus and Acb shell (Vertes, 2004; Gabbott et al., 2005), and these cortical areas contain considerable densities of opioid peptides and their receptors (Mansour et al., 1987; Martin-Schild et al., 1999; Leriche et al., 2007). While numerous imaging studies have shown frontal regions to display strong activation in response to food-related cues in humans (Gottfried et al., 2003; Wang et al., 2004; Felsted et al., 2010), little is known of the role of PFC in driving basic feeding responses. The PFC guides the execution of goal-directed behaviors, including feeding, based upon online representations of complex stimulus–reinforcement relationships (Schoenbaum et al., 2003; Petrovich et al., 2007). Yet, it remains unclear whether there are specific PFC subregions that provoke the initiation of basic feeding responses when feeding drive is low (as with *ad libitum*-fed animals offered standard chow), rather than just modulate feeding (as evidenced by reorganization of ingestive behavior that has been elicited by a homeostatic drive or high-incentive food). Furthermore, the behavioral effects of local opioid stimulation, which appears to have a somewhat "privileged" role in eliciting feeding from select subcortical structures, have never been studied in the PFC. Such information could have clinical relevance because frontal areas exhibit aberrant patterns of activity in response to food stimuli in individuals with anorexia or bulimia nervosa (Uher et al., 2003, 2004).

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To address whether opioids act within the PFC to drive feeding behavior, we undertook a microinfusion mapping study in frontal cortical subregions using specific opioid receptor agonists. We were particularly interested in exploring the selectivity of local opioid stimulation by comparing effects obtained with opioid peptides to those obtained by local stimulation or blockade of cortical monoamines. In the course of these studies, we discovered μ-opioid-sensitive frontal cortical "feeding hotspots" in the ventral medial PFC (vmPFC) and orbitofrontal cortex of the rat, and investigated the behavioral and pharmacological specificity, and food-type selectivity, of feeding responses produced by manipulating these sites.

Materials and Methods

All procedures were evaluated and approved by the Institutional Animal Care and Use Committee of the University of Wisconsin-Madison, and are in accordance with the guidelines promulgated in the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Facilities have been approved by the American Association for the Accreditation of Laboratory Animal Care.

Subjects. Male Sprague Dawley rats, obtained from Harlan, weighing 275–290 g upon arrival in the laboratory, were used in all experiments. Rats were pair-housed in clear polycarbonate cages (9.5 inch width × 17 inch length × 8 inch height), with cob bedding, in a light- and temperature-controlled vivarium. Animals were maintained under a 12 h light/dark cycle (lights on at 7:00 A.M.). Food (rat-chow pellets, Agway) and water were available *ad libitum*, except during food restriction phases and according to the dictates of the experiments as described in the Experimental design section. Animals were handled gently daily to reduce stress. Subjects were tested between the hours of 11:00 A.M. and 4:00 P.M, during the light phase of the animals' dark/light cycle.

Surgical procedures. Rats (weighing 300–330 g at the time of surgery) were anesthetized with a xylazine/ketamine mixture (intraperitoneal injection of 13 mg/kg xylazine and 87 mg/kg ketamine) or with isofluorane gas and secured in a Kopf stereotaxic frame. The toothbar was set at 4.0 mm below the interaural line for all surgeries. Bilateral stainless steel cannulas (10 mm long, 23 gauge) were implanted according to standard stereotaxic procedures. For the vmPFC placements, cannulas were placed at an acute angle to the midline to avoid damage to the medial wall of the cortex. For the vmPFC, coordinates of the injection site were as follows: anteroposterior (AP), +3.0 mm from bregma; mediolateral (ML), ±2.1 mm from midline; dorsoventral (DV), −5.2 mm from skull surface with cannulas angled at 19° from vertical. Angled placements were not used for the primary motor cortex (M1) and ventral orbital cortex (voC). For M1, the coordinates were as follows: AP, + 2.5 from bregma; ML, ±2.8 mm from midline; DV, −2.3 from the skull surface. For the voC, the coordinates were as follows: AP, +3.0 mm from bregma; ML, ±2.5 mm from midline; DV, −5.4 mm from skull surface. Cannulas were fixed in place 2.5 mm above the target sites (for vmC) or 1 mm above the target sites (for M1 and voC) with dental acrylic (New Truliner) and anchoring skull screws (Plastics One). Wire stylets (10 mm long, 30 gauge) were placed in the cannulas to prevent blockage. Rats were given an intramuscular injection of penicillin (0.3 ml of a 300,000 U/μl suspension; Phoenix Pharmaceuticals), placed in heated recovery cages, returned to their home cages upon awakening, and given a recovery period of no less than 5 d (with daily health checks) before behavioral testing commenced.

Drugs. DAMGO ([D-Ala2, N-Me-Phe4, Gly5-ol]-enkephalin), dopamine hydrochloride, serotonin hydrochloride, norepinephrine hydrochloride, SCH23390 (7-chloro-3-methyl-1-phenyl-1,2,4,5-tetrahydro-3-benzazepin-8-ol), raclopride, WAY100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]- *N*-(2-pyridyl)cyclohexanecarboxamide), ICI118551 [[3-(isopropylamino)-1-[[(7-methyl-4-indanyl)oxy]butan-2-ol], betaxolol hydrochloride, LPK-26 [[(2-(3,4-dichloro)-phenyl)-N-methyl-N-[(1S)-1-(2-isopropyl)-2-(1-(3-pyrrolinyl))ethyl] acetamides], and muscimol were all obtained from Sigma-Aldrich. For dopamine, serotonin, and norepinephrine, the racemic salts were used. MDL-11,939 [α-Phenyl-1-(2-phenylethyl)-4-piperidinemethanol] was obtained from Tocris Bioscience. DPDPE ([d-Pen2,d-Pen5]enkephalin) and prazosin were obtained from Bachem.

Microinfusion procedures. For intracerebral microinfusions, rats were held gently and stylets were removed from the guide cannula. Stainless steel injectors, connected via polyethylene tubing (PE-10, Becton Dickinson) to 10 μl capacity Hamilton syringes on a Harvard microdrive pump, were lowered to the site of infusion. The flow rate for infusions was 0.32 μl/min. The total infusate volume for the bilateral infusions was 0.5 μl per side. After infusions, injectors were left in place for an additional minute to allow for diffusion of the injectate into the tissue. Injectors were then removed, and wire stylets replaced.

Behavioral testing procedures. Behavioral testing was performed in clear polycarbonate cages (9.5 inch width × 17 inch length × 8 inch height) with wire-grid floors. Experiments were conducted with either food-deprived rats or *ad libitum*-maintained rats given preweighed quantities of chow pellets on the cage floors, *ad libitum*-maintained rats given preweighed quantities of carbohydrate- or fat-enriched test diets, or with water-deprived rats given water but not food. For the chewing/gnawing experiment, *ad libitum*-fed animals were presented with wood blocks that were similar in size and shape to standard chow pellets instead of standard chow. Water was available in all testing conditions from an overhead graduated burette. Animals were given intracerebral infusions, as described above, and placed immediately into the testing cages for 30 or 60 min testing sessions. During testing, rats were rated by an experimenter blind to treatment, using an event recorder interfaced to a personal computer. The following behaviors were monitored: cage crossings, ambulation across the midpoint of the cage's long axis; rears, rearing up on the hind legs; grooming, cleansing of the face, paws, and body; feeding, uninterrupted bouts of chow-pellet intake; gnawing, uninterrupted bouts of wood chip chewing; and drinking, uninterrupted bouts of water intake. The frequency and duration of all these behaviors were recorded, except for cage crossings, for which only frequency was recorded. To record the duration of a given behavioral event, a switch was depressed on the event recorder that started an automatic timer (specific for that behavior) at the initiation of the behavior. The timer was switched off when the behavior was interrupted by a different behavior (e.g., a rat engaged in a drinking bout stopping his drinking to rear or ambulate across the cage). From the frequency and duration data, the overall mean duration of each rearing, feeding, wood chip chewing, or drinking bout was calculated. Uneaten food and food spillage were recorded for chow intake experiments. The volume of water ingested was recorded for all experiments.

Experimental design. After at least 5 d of recovery from surgery (during which rats were handled and given daily health checks), animals were accommodated for 2 d to a food restriction schedule and a water deprivation schedule, or maintained on an *ad libitum*-fed schedule, as determined by the dictates of the experiment. Where used, the food restriction schedule consisted of food being taken away overnight, 18 h before the next day's testing session, and replaced upon completion of the session. This feeding schedule was maintained throughout the experiment. Where used, the water deprivation schedule consisted of water being removed 15 h before the next day's testing session and replaced upon completion of testing, with this schedule persisting for the duration of the study.

All rats were given "sham injections," in which microinfusion injectors were lowered into the infusion cannula but not into the brain. The next day, all rats were given saline injections into the brain and placed into the testing cages (described in Behavioral testing procedures, above) with both chow pellets and water bottles (for *ad libitum*-fed or food-restricted animals) for either 30 min (monoamine antagonist studies) or 60 min (all other experiments). Water-deprived animals were presented with only water bottles. These procedures served to habituate the rats to microinfusion and testing.

Two days later, drug testing commenced. For the feeding microstructure studies, *ad libitum*-fed ($n = 8$) or food-deprived ($n = 8$) rats received intra-vmPFC infusions of DAMGO (0, 0.25, and 2.5 μg/0.5 μl). An additional food-deprived cohort ($n = 8$) received intra-vmPFC infusions of muscimol (0, 0.75 ng/0.5 μl). For the microinfusion mapping study, separate groups of *ad libitum*-maintained rats were injected with

Table 1. Composition of diets used in food choice experiment

	Diet	
	Carbohydrate/protein	Fat/protein
Casein[a]	195.0	329.0
L-Cystine	3.0	4.9
Corn starch	440.63	
Dextrin	210.0	
Sucrose	70.0	
Vegetable shortening, hydrogenated (Primex)		441.0
Safflower oil, linoleic		77.0
Cellulose	40.0	77.49
Mineral mix, calcium phosphate deficient (79055)[b]	13.37	22.7
Calcium phosphate, dibasic	9.25	16.0
Calcium carbonate	6.25	10.6
Vitamin mix, AIN-76A (40077)[b]	10.0	17.0
Choline bitartrate	2.5	4.25
TBHQ, antioxidant		0.06
Weight	1000	1000
Total energy (kcal)[c]	3400	5900
Energy density (kcal/g)	3.4	5.9

All components are expressed as weight (in grams, unless otherwise indicated). TBHQ, Tert-butylhydroquinone.

[a]Assuming a protein content of 90%.

[b]The vitamin and mineral mixes contain 97 and 12% sucrose, respectively.

[c]Based on energy values of 4, 9, and 4 kcal/g for carbohydrate, fat, and protein, respectively.

DAMGO into either the voC (0, 0.25, and 2.5 μg/0.5 μl, $n = 8$) or M1 (0, 0.25, and 2.5 μg/0.5 μl, $n = 6$). For the experiments investigating pharmacological specificity, separate groups of *ad libitum*-fed rats were given intra-vmPFC infusions of DPDPE (0, 0.31, and 3.1 μg/0.5 μl, $n = 8$), LPK-26 (0, 0.16, and 1.6 μg/0.5 μl, $n = 8$), dopamine hydrochloride (0, 3.8, 12.70, and 38.0 μg/0.5 μl, $n = 8$), serotonin hydrochloride (0, 4.25, 14.2, and 42.5 μg/0.5 μl, $n = 8$), and norepinephrine hydrochloride (0, 4.12, 13.7, and 41.2 μg/0.5 μl, $n = 8$). Food-deprived rats were given infusions of SCH23390 (0, 1.0, and 2.0 μg/0.5 μl, $n = 8$), raclopride (0, 1.0, and 2.0 μg/0.5 μl, $n = 7$), WAY100635 (0 and 2.0 μg/0.5 μl, $n = 4$), MDL-11,939 (0, 0.15, and 0.45 μg/0.5 μl, $n = 11$), prazosin (0, 0.25 μg/0.5 μl, $n = 4$), or a mixture of betaxolol/ICI118551 (0, and 1/1 μg/0.5 μl, $n = 4$). For the behavioral control experiments, *ad libitum*-fed rats ($n = 8$) received intra-vmPFC DAMGO infusions and were allowed to chew on wood blocks instead of food pellets. The wood blocks resembled standard chow in both size and shape. For the drinking experiment, water-deprived animals ($n = 8$) received intra-vmPFC DAMGO infusions and were allowed to drink water but not to chew/ingest food pellets, as food was not included in the testing cages. For all experiments, unconditioned motor and ingestive behaviors were monitored and recorded for 30 min (monoamine antagonist studies and muscimol experiment) or 60 min (all other experiments) as described in Behavioral testing procedures. Each rat received every dose according to within-subjects Latin square designs. Testing days were separated from each other by at least 1 interim day on which no drug infusions or behavioral testing occurred.

Food choice experiment. We performed an experiment to test the effect of intra-vmPFC μ-opioid stimulation on the choice between two palatable test diets differing in macronutrient composition (i.e., fat-enriched vs carbohydrate-enriched test diets), because previous studies have suggested that the hyperphagia induced by stimulation of subcortical μ-opioid systems exhibits some specificity for palatable, fat-enriched foods. Thus, we were interested in evaluating whether the same holds true for frontal cortical μ-opioid stimulation. The test diets and overall experimental procedures were identical to those described by Zhang et al. (1998); this study demonstrated that μ-opioid stimulation in the nucleus accumbens produces a fat preference.

As indicated in Table 1, the carbohydrate- and fat-enriched test diets contained equal amounts of vitamins, minerals, choline chloride, fiber, and protein when equated on a caloric basis, but the diets differed with regard to the content of simple and complex carbohydrates, and fat. The carbohydrate-enriched diet contained sucrose, and was therefore sweeter than the fat-enriched diet. Diets were obtained from Teklad Diets. There was a 5 d acclimation period in which both test diets (placed in glass jars affixed to the cage floor grids) were presented for 1 h daily in the testing cages described above in Behavioral testing procedures. The positions of the jars were reversed daily to prevent the development of location biases. At the end of each acclimation session, rats were removed to their home cages where they had *ad libitum* access to standard rat chow, diet jars were removed and weighed, and corresponding gram intake was calculated and corrected for spillage. Gram intake values were used to calculate total caloric intake (the energy density of the carbohydrate-enriched diet was 3.4 kcal/g, that of the fat-enriched diet, 5.9 kcal/g).

After the rats were adapted to the testing diets, baseline intakes over 1 h were measured on 3 consecutive days. Based on these data, rats were divided into carbohydrate-preferring (CP) and fat-preferring (FP) groups on the basis of the ratio of the average of carbohydrate intake to fat intake. For the CP group, the ratio was >1 on each of 3 consecutive days; for the FP group, the ratio was <1 on 3 consecutive days. A total of 20 rats were used for this study; 8 were determined to have a baseline carbohydrate preference, and 6, a fat preference.

One day after the determination of the baseline preferences, testing with DAMGO commenced. Rats were placed in the testing cages with both diets present immediately after intra-vmPFC infusion of one of three DAMGO doses (0, 0.25, or 2.5 μg/0.5 μl). Each rat received every dose according to a Latin square design; 1 drug-free and testing-free interim day separated each test day. The side of the cage where each diet was placed was counterbalanced across rats and treatments. Once this phase of the experiment was complete, animals received an additional treatment of 2.5 μg/0.5 μl DAMGO or saline directly into the vmPFC and were allowed to consume either the fat-enriched or the carbohydrate-enriched diet presented alone.

Verification of placements. At the end of each experiment, rats were deeply anesthetized with sodium pentobarbital or isofluorane and perfused transcardially with a 0.9% saline solution followed by 10% formalin in phosphate buffer. Brains were collected and stored in 10% formalin. Coronal sections (60 μm) were cut through the infusion site on a cryostat microtome, collected on slides, stained with cresyl violet, and subsequently reviewed to verify correct placement of the intracortical injections. Images of representative sections from each experiment were captured using Scion Image software on a computer interfaced with a microscope-mounted Hitachi HV-C20 CCD camera.

Statistical analyses. Data scores for each behavioral measure were subjected to one-factor (dose) repeated-measures ANOVAs. Preplanned contrasts were used to make individual comparisons among vehicle and each drug dose. We report the results of these contrasts in cases where there was a significant main effect of drug in the ANOVA (α level, $p < 0.05$).

Results

Intra-PFC DAMGO infusions produced hyperphagia and a "fragmented" motor profile

To determine whether opioids in the PFC play a role in modulating food intake and organizing feeding microstructure, we infused DAMGO into vmPFC in both *ad libitum*-fed and food-deprived animals. As shown in Figure 1*A*, DAMGO infusions into the vmPFC increased food intake significantly in both *ad libitum*-fed animals ($F_{(2,12)} = 51.59$, $p < 0.0002$) as well as in food-deprived rats ($F_{(2,14)} = 19.33$, $p < 0.0002$). For both conditions, preplanned contrasts among individual means indicated a significant effect between vehicle and the high DAMGO dose ($p < 0.0002$ for both). Figure 1*B* shows that the total number of feeding bouts initiated was significantly increased by infusion of DAMGO into vmPFC for both *ad libitum*-fed animals ($F_{(2,12)} = 19.16$, $p < 0.0003$) and for food-deprived animals ($F_{(2,14)} = 12.42$, $p < 0.0009$). Preplanned contrasts revealed significant differences between vehicle and both doses of DAMGO ($p < 0.05$ for low dose; $p < 0.0003$ for high dose) in the food-deprived condition. A significant increase in total eating duration was also observed for both conditions (*ad libitum*-fed animals: $F_{(2,12)} = 23.64$, $p < 0.0002$; food-deprived animals: $F_{(2,14)} = 3.80$, $p < 0.05$) (Fig. 1*C*)

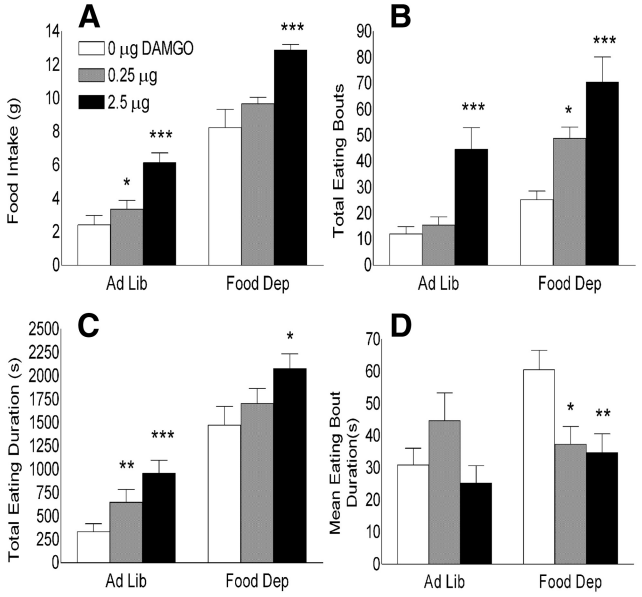

Figure 1. Microanalysis of eating after DAMGO infusion into vmPFC in *ad libitum*-fed and food-deprived animals. ***A–D***, Shown are effects on total food intake over 60 min (***A***), total number of eating bouts in the entire test session (***B***), total time spent eating during the entire session (***C***), and average duration of each eating bout (***D***). Values represent mean ± SEM for each treatment. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with vehicle (from preplanned contrasts). DAMGO doses are in micrograms per 0.5 μl per side. Ad Lib, *Ad libitum*-fed rats; Food Dep, food-deprived rats.

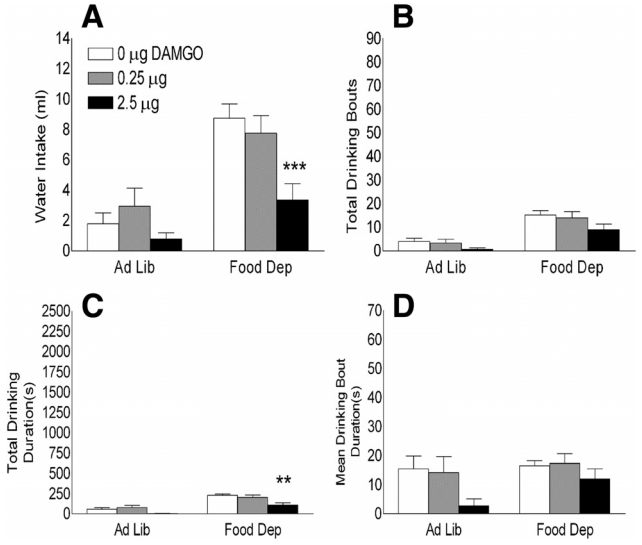

Figure 2. Microanalysis of drinking after DAMGO infusion into vmPFC in *ad libitum*-fed and food-deprived animals. ***A–D***, Shown are effects on total water intake over 60 min (***A***), total number of drinking bouts in the entire test session (***B***), total time spent drinking during the entire session (***C***), and average duration of a drinking bout (***D***). Values represent mean ± SEM for each treatment. **$p < 0.01$, ***$p < 0.001$, compared with vehicle (from preplanned contrasts). DAMGO doses are in micrograms per 0.5 μl per side. Ad Lib, *Ad libitum*-fed rats; Food Dep, food-deprived rats.

following DAMGO infusions into vmPFC. Finally, while no main effect was detected in the *ad libitum* condition for mean eating bout duration ($F_{(2,12)} = 2.23$, NS), there was a significant DAMGO-induced decrease in this measure for food-deprived animals ($F_{(2,14)} = 6.58$, $p < 0.01$) (Fig. 1*D*). Preplanned contrasts revealed significant differences between vehicle and both doses of DAMGO ($p < 0.05$ for low dose; $p < 0.006$ for high dose). Together, these data indicate that DAMGO-treated rats increased overall food intake by engaging in numerous, brief feeding bouts.

As shown in Figure 2*A*, DAMGO infusions into the vmPFC decreased water intake only in food-deprived animals ($F_{(2,14)} = 10.33$, $p < 0.002$; preplanned contrasts, vehicle vs high dose of DAMGO, $p < 0.001$; for *ad libitum*-fed, $F_{(2,12)} = 1.84$, NS). These data suggest that the decrease in water intake in food-deprived animals may have been due to behavioral competition between drinking and the high rates of feeding in the food-deprived rats. The only significant DAMGO-induced effect on drinking microstructure was a significant decrease in total drinking duration for both *ad libitum*-fed and food-deprived animals ($F_{(2,12)} = 3.40$, $p < 0.05$; $F_{(2,14)} = 9.21$, $p < 0.003$, respectively) (Fig. 2*C*). There were trends toward decreased total number of drinking bouts initiated, although these did not achieve statistical significance (*ad libitum*-fed: $F_{(2,12)} = 2.94$, NS; food-deprived: $F_{(2,14)} = 3.10$, NS) (Fig. 2*B*) and mean drinking bout duration (*ad libitum*-fed: $F_{(2,12)} = 2.99$, NS; food-deprived: $F_{(2,14)} = 2.42$, NS) (Fig. 2*D*).

Intra-vmPFC DAMGO infusions increased ambulatory counts significantly in *ad libitum*-fed animals and produced a strong trend toward an increase in food-deprived animals (*ad libitum*-fed: $F_{(2,12)} = 18.60$, $p < 0.0003$; food-deprived: $F_{(2,14)} = 3.58$, $p = 0.0554$) (Fig. 3*A*). Rearing counts were not affected by intra-vmPFC infusions of DAMGO in *ad libitum*-fed animals ($F_{(2,12)} = 0.01$, NS), but there was an increase in food-deprived animals ($F_{(2,14)} = 8.77$, $p < 0.004$) (Fig. 3*B*) characterized by an inverted u-shaped dose-response profile. Preplanned contrasts indicated a significant effect between vehicle and the low DAMGO dose ($p < 0.005$), and between the low and high DAMGO doses ($p < 0.002$). Furthermore, there was a significant, dose-dependent reduction in mean rearing bout duration for both *ad libitum*-fed and food-deprived animals ($F_{(2,12)} = 14.20$, $p < 0.0008$; $F_{(2,14)} = 16.55$, $p < 0.0003$, respectively) (Fig. 3*B*, inset). Grooming counts were not affected by intra-vmPFC infusions of DAMGO in *ad libitum*-fed animals ($F_{(2,12)} = 0.40$, NS), but there was a small increase at the lower dose in food-deprived animals ($F_{(2,14)} = 4.06$, $p < 0.05$) (Fig. 3*C*). A dramatic reduction in mean grooming bout duration for both *ad libitum*-fed and food-deprived animals was also observed ($F_{(2,12)} = 10.68$, $p < 0.003$; $F_{(2,14)} = 4.25$, $p < 0.05$, respectively) (Fig. 3*C*, inset). Hence, the overall pattern of DAMGO effects on these noningestive behaviors was to dose-dependently augment horizontal activity and shorten the duration of rearing and grooming behaviors, once initiated.

Muscimol-induced inactivation of the vmPFC affects ambulatory activity and feeding bout duration in a manner opposite to DAMGO

To explore the effect of temporarily inactivating the vmPFC on feeding microstructure, we injected muscimol into this site in food-deprived animals. The dose of muscimol used (75 ng) has been shown to markedly suppress electrophysiological activity in the PFC (C. Berridge, personal communication). Opposite in direction to the effects of DAMGO, infusion of muscimol into the vmPFC resulted in a reduction in locomotor activity ($F_{(1,6)} = 6.11$, $p < 0.05$) (see Table 3), a reduction in the number of feeding bouts initiated (data not shown), and a prolongation of the average duration of each individual feeding bout ($F_{(1,6)} = 32.90$, $p < 0.01$) (see Table 3). Interestingly, food intake was left unaffected, suggesting that the global feeding rate was unaltered by cortical inactivation (see Table 3). Collectively, these results show that temporary pharmacological inactivation of the vmPFC reduces

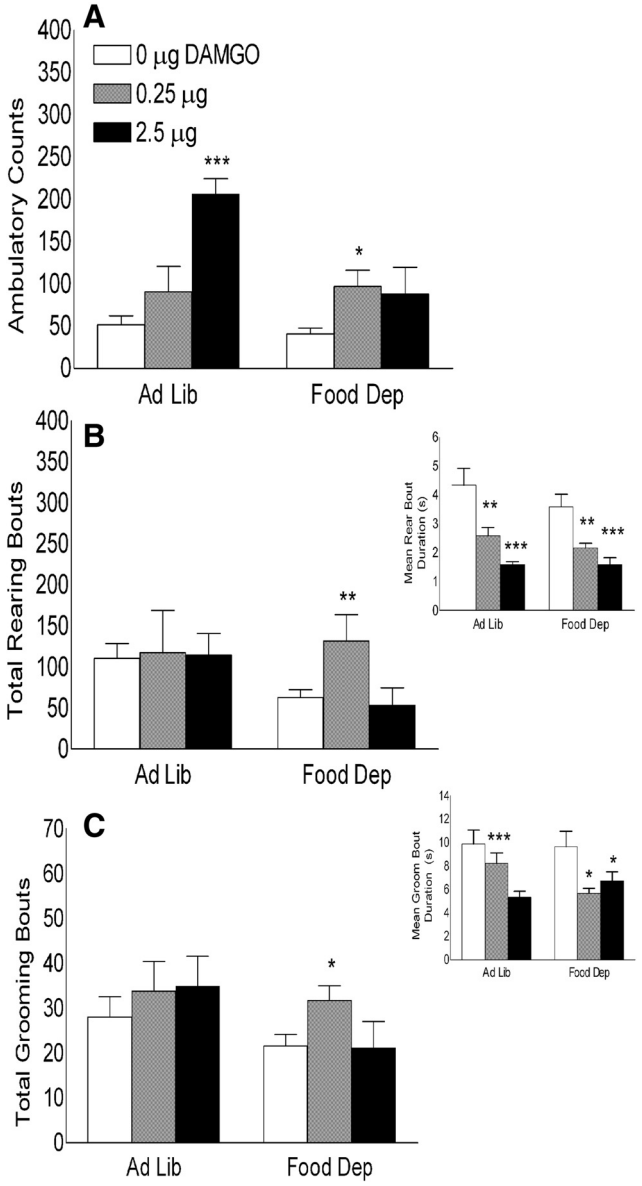

Figure 3. ***A–C***, Effects of DAMGO infusion into vmPFC on noningestive behaviors over a 60 min test session: total number of cage crossings (***A***); total number of rearing bouts (***B***); total number of grooming bouts (***C***). For ***B*** and ***C***, insets depict mean duration of individual rearing and grooming bouts, respectively. Values represent mean ± SEM for each treatment. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with vehicle (from preplanned contrasts). DAMGO doses are in micrograms per 0.5 μl per side. Ad Lib, *Ad libitum*-fed rats; Food Dep, food-deprived rats.

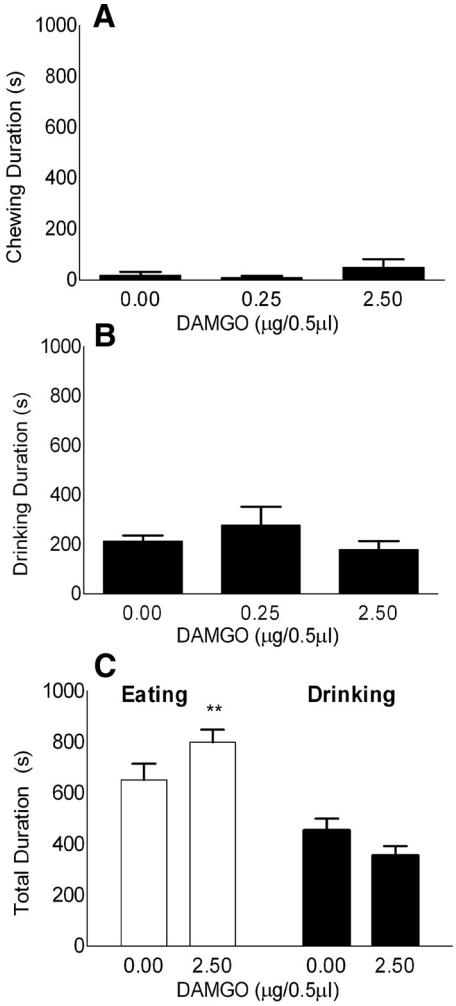

Figure 4. ***A–C***, Effects over a 60 min test session of intra-vmPFC DAMGO infusion on wood chip chewing/gnawing (***A***), deprivation-induced water drinking (***B***), and ingestive behavior in water-deprived rats given a concurrent choice to eat and drink (***C***). Values represent mean ± SEM for each treatment.**$p < 0.01$, compared with vehicle. DAMGO doses are in micrograms per 0.5 μl per side.

exploratory behavior and prolongs individual feeding bouts, a behavioral profile opposite to that observed with DAMGO infusions into the same area.

Intra-vmPFC DAMGO did not elicit nonspecific gnawing or enhance deprivation-induced water intake

To determine whether the DAMGO-associated feeding effects were due to nonspecific oral behaviors directed at the food rather than an induction of ingestive behavior itself, we infused DAMGO into the vmPFC of *ad libitum*-fed animals given the opportunity to chew on wood pellets (instead of food). In addition, to determine whether the increase in ingestive behavior was specific to feeding or was generalizable to other ingestive behaviors, like drinking, we infused DAMGO into the vmPFC of water-deprived rats. As shown in Figure 4*A*, DAMGO infusions into the vmPFC failed to affect the number of chewing bouts in *ad libitum*-fed animals given the opportunity to chew on wood pellets ($F_{(2,14)} = 1.01$, NS). Nevertheless, intra-vmPFC infusions of DAMGO significantly increased ambulatory counts ($F_{(2,14)} = 7.97$, $p < 0.005$), indicating that the drug was active in these animals. Furthermore, as shown in Figure 4*B*, DAMGO infusions into the vmPFC failed to significantly affect the number of drinking bouts in water-deprived animals ($F_{(2,14)} = 1.14$, NS). Furthermore, total water intake was also unaffected by intra-vmPFC DAMGO infusions ($F_{(2,14)} = 0.88$, NS). Again, ambulatory counts were increased in these water-deprived animals ($F_{(2,14)} = 25.70$, $p < 0.0002$), providing a positive indication of pharmacological activity. Preplanned contrasts among individual means indicated a significant effect on ambulation between vehicle and both doses of DAMGO ($p < 0.003$ for low doses; $p < 0.0002$ for high doses).

Finally, water deprivation produces a mild anorexia, thereby producing a "mixed" drive to drink and eat. We took advantage of this motivational condition to carry out a more stringent test of the specificity of intra-vmPFC DAMGO by placing water-deprived rats in a choice situation with both food and

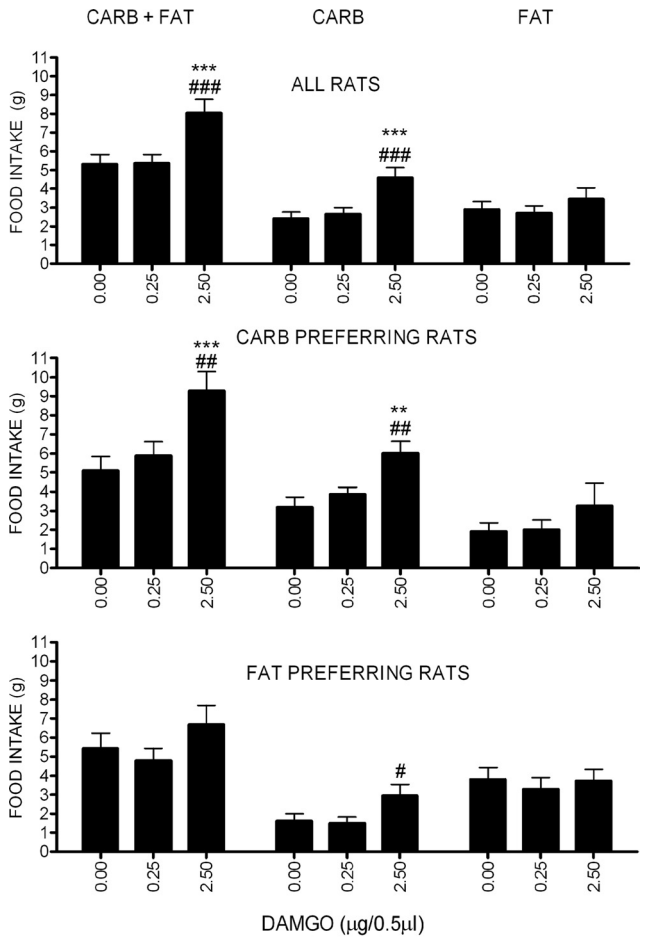

Figure 5. One-hour cumulative intake of diets after intra-vmPFC injection of vehicle or DAMGO when carbohydrate- or fat-rich diets were presented to rats concurrently. Top, effect of the treatments on diet selection when all of the rats were considered as one group. Middle and bottom, Intake when rats were divided into carbohydrate-preferring and fat-preferring groups, respectively. See text for method of grouping. Values represent means ± SEM. $^{**}p < 0.01$, $^{***}p < 0.001$, compared with vehicle (from preplanned contrasts). #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$, compared with low dose. DAMGO doses are in micrograms per 0.5 μl per side.

water present. Figure 4*C* shows that intra-vmPFC DAMGO (2.5 μg/0.5 μl) infusions significantly increased total eating duration ($F_{(1,7)} = 16.30$, $p < 0.005$), while leaving total drinking duration unaffected.

Together, these studies demonstrate that DAMGO-induced hyperphagia appears to be a feeding-specific motivational state; the results cannot be attributed to nonspecific oral behavior or nonselective motivational effects.

Intra-vmPFC DAMGO selectively augments carbohydrate intake in a choice test

We performed a test of the effects of intra-vmPFC DAMGO when rats were confronted with a concurrent choice between palatable carbohydrate- versus fat-enriched test diets (for details, see Materials and Methods). As depicted in the top panel of Figure 5, DAMGO infusions into the vmPFC significantly increased total food intake when the carbohydrate- and fat-enriched diets were summed ($F_{(2,19)} = 16.22$, $p < 0.001$, main effect of DAMGO dose). Comparisons among means with Bonferroni-corrected *t* tests revealed a significant effect between vehicle and the high DAMGO dose, and between the low and high DAMGO doses (α level ≤ 0.017). This effect on total intake was mainly due to an

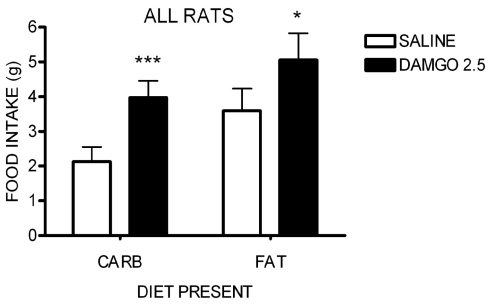

Figure 6. The effect of intra-vmPFC injection of vehicle and 2.5 μg/0.5 μl dose of DAMGO on fat and carbohydrate (CARB) intake when these two diets were presented to rats separately for 1 h. Values represent means ± SEM. $^{*}p < 0.05$, $^{***}p < 0.001$, compared with vehicle (from preplanned contrasts). DAMGO doses are in micrograms per 0.5 μl per side.

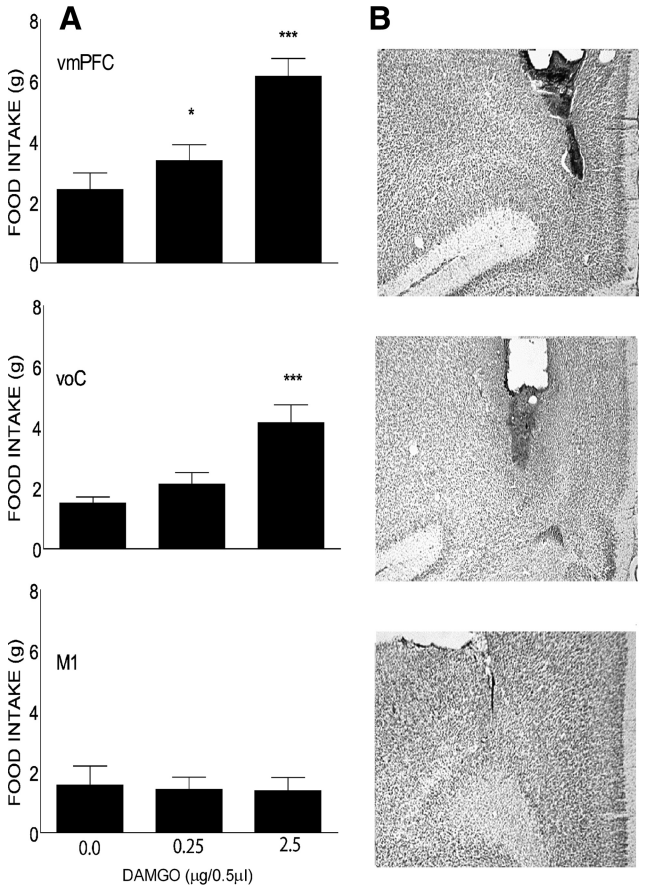

Figure 7. Anatomical specificity of DAMGO-induced effects. ***A***, Food intake effects following infusion of DAMGO into vmPFC, voC, and M1. Values represent mean ± SEM. for each treatment. $^{*}p < 0.05$, $^{***}p < 0.001$, compared with vehicle (from preplanned contrasts). DAMGO doses are in micrograms per 0.5 μl/side. ***B***, Representative photomicrographs of injector placements into vmPFC, voC, and M1.

increase in intake of the carbohydrate-enriched diet. Thus, when intake was analyzed separately for each of the two diets, a significant effect was found only for the carbohydrate-enriched food (carbohydrate-enriched diet: $F_{(2,19)} = 14.73$, $p < 0.001$; fat-enriched diet: $F_{(2,19)} = 2.00$, NS) (Fig. 5, top). As with total intake, significant effects were seen only with the higher DAMGO dose (Fig. 5, means comparisons).

As shown in Figure 5, DAMGO-induced intake and macronutrient preference were also analyzed after separating the rats into CP or FP subgroups based on baseline food preferences (for

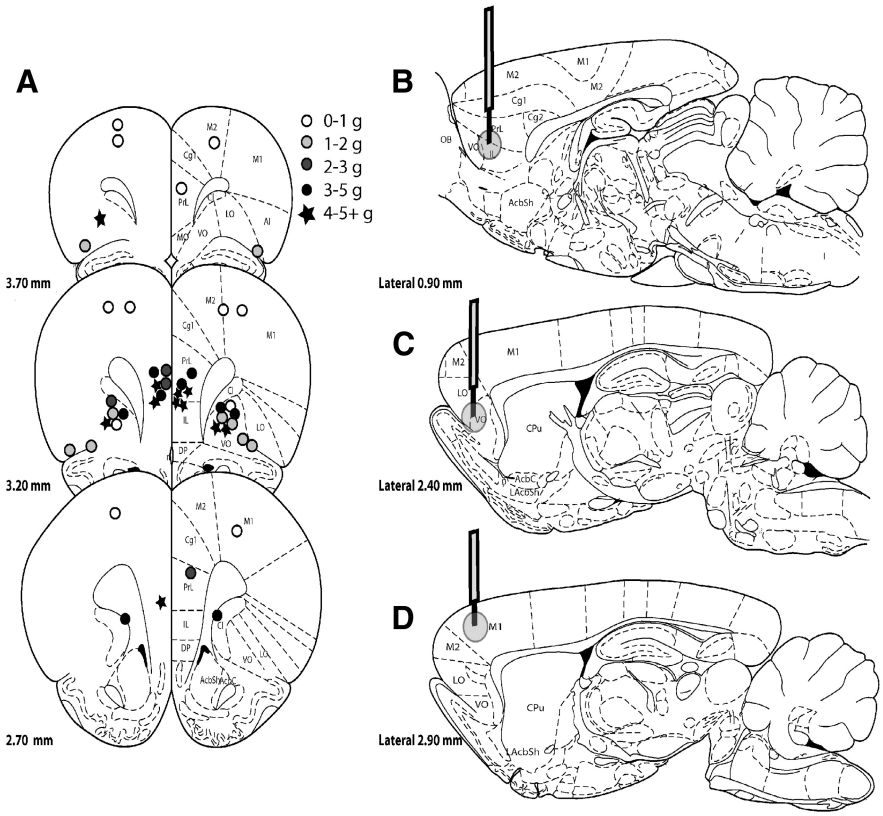

Figure 8. Schematic diagrams showing injection sites into frontal cortical regions. ***A***, Coronal sections of animals receiving DAMGO infusion into vmPFC, voC, and M1. Values represent difference in gram intake between vehicle and high DAMGO dose infusion. ***B–D***, Also shown are sagittal sections depicting relative injectate spread following intracranial infusions into vmPFC (***B***), voC (***C***), and M1 (***D***). Line drawings are adapted from Paxinos and Watson (1998) with permission. PrL, Prelimbic cortex; MO, medial orbital cortex; VO, ventral orbital cortex; LO, lateral orbital cortex; AI, agranular insular cortex; M2, secondary motor cortex; Cg1, cingulate cortex, area 1; IL, infralimbic cortex; DP, dorsal peduncular cortex; AcbSh, accumbens nucleus, shell; AcbC, accumbens nucleus, core; Cl, claustrum; CPu, caudate putamen; Cg2, cingulate cortex, area 2; OB, olfactory bulb.

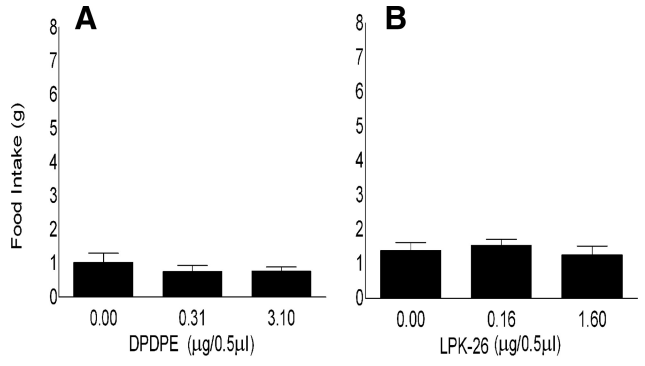

Figure 9. ***A***, ***B***, Effects over a 60 min test session of intra-vmPFC DPDPE (***A***) and LPK-26 (***B***) on food intake in *ad libitum*-fed rats. Values represent mean ± SEM for each treatment. Drug doses are in micrograms per 0.5 μl per side.

details, see Materials and Methods). Carbohydrate-preferring animals exhibited a statistically significant increase in total (i.e., carbohydrate + fat) food intake ($F_{(2,7)} = 10.42$, $p < 0.01$). This effect was seen mainly at the high DAMGO dose (for specific comparisons, see Fig. 5). When the two diets were considered separately, carbohydrate-preferring animals increased their total intake of the carbohydrate-enriched, but not fat-enriched, diet (carbohydrate-enriched diet: $F_{(2,7)} = 9.48$, $p < 0.01$; fat-enriched diet: $F_{(2,8)} = 2.22$, NS) (for specific comparisons, see Fig. 5). Interestingly, and in contrast to effects observed after DAMGO infusions into the nucleus accumbens (Zhang et al., 1998), DAMGO infusions into the vmPFC increased carbohydrate-enriched diet intake in fat-preferring animals ($F_{(2,6)} = 5.32$, $p < 0.05$). *Post hoc* analyses with Bonferroni-corrected *t* tests revealed significant differences between vehicle and the high DAMGO dose. In summary, when rats are presented with a choice between palatable carbohydrate-enriched versus fat-enriched test diets, DAMGO infusions into the vmPFC selectively augment intake of the carbohydrate-enriched diet, even in rats with a baseline fat preference.

When the carbohydrate- or fat-enriched test diets were presented individually, DAMGO infusions into the vmPFC increased intake of both the carbohydrate-enriched diet ($F_{(1,11)} = 24.56$, $p < 0.001$) and the fat-enriched diet ($F_{(1,11)} = 4.94$, $p < 0.05$), as shown in Figure 6. Unfortunately, this experimental manipulation was not run on a sufficient number of rats to parse out effects on fat-preferring versus carbohydrate-preferring rats. Nevertheless, the significant DAMGO-induced augmentation of fat intake when this test diet was presented alone strongly suggests that the lack of effects on fat intake in the choice situation was due neither to the use of an insufficiently high DAMGO dose nor to intra-vmPFC DAMGO-induced avoidance of fat-enriched foods.

DAMGO-induced feeding effects were specific to vmPFC and ventral orbital cortex

To investigate whether the DAMGO-induced behavioral effects are specific to the vmPFC, or generalizable to other regions of frontal cortex, we infused DAMGO into voC and M1. As shown in Figure 7*A*, DAMGO infusions into the voC increased food intake significantly in *ad libitum*-fed animals ($F_{(2,14)} = 14.40$, $p < 0.0005$). In contrast, DAMGO infusions into M1 failed to affect food intake in *ad libitum*-fed animals ($F_{(2,10)} = 0.18$, NS). Representative micrographs of injector placements into vmPFC, voC, and M1 are shown in Figure 7*B*. As can be seen, the cannula and injector tracks are clearly visible with no unusual damage in the targeted areas. Placements within vmPFC were confined to the infralimbic/prelimbic boundary. Figure 8*A* shows a schematic diagram summarizing injector tip placements into vmPFC, voC, and M1, along with the difference in gram food intake between vehicle infusion and high DAMGO dose infusion plotted for each respective placement. The most consistent area to yield strong to very strong effects was the vmPFC, with positive effects seen in both infralimbic and prelimbic cortex. Moderate to strong effects were produced from voC. A rostrocaudal gradient was also detected, with weak effects from anterior levels of vmPFC. The effect spread in voC was much more homogenous, with placements yielding low, moderate, and very strong effects sizes intermingled within a relatively restricted region of dorsal voC. Weak effects were observed from lateral voC placements. Sagittal sections showing approximate diffusion of injectate from injections

Table 2. Ingestive and exploratory behaviors following administration of opioid receptor-specific agonists into vmPFC

	Mean eat bout duration (s)	Water intake (ml)	Mean drink bout duration (s)	Locomotor counts	Rearing counts
DPDPE (μg/0.5 μl)					
0	24.7 ± 9.57	0.8 ± 0.37	8.0 ± 1.72	47.4 ± 8.47	106.0 ± 33.87
0.31	24.9 ± 12.19	0.8 ± 0.41	7.8 ± 3.81	65.3 ± 13.02	132.1 ± 34.61
3.10	14.7 ± 3.23	0.8 ± 0.35	2.9 ± 1.00	69.9 ± 15.24	153.2 ± 47.11
LPK-26 (μg/0.5 μl)					
0	18.0 ± 3.12	0.9 ± 0.39	7.6 ± 1.97	64.6 ± 7.32	117.4 ± 19.23
0.16	19.1 ± 1.73	1.2 ± 0.48	8.5 ± 3.54	63.6 ± 7.41	118.1 ± 19.78
1.60	76.4 ± 30.55*	0.0 ± 0.00	0.0 ± 0.00*	35.5 ± 9.75*	62.1 ± 21.63

Values represent mean ± SEM. *$p < 0.05$, compared with vehicle.

into vmPFC (Fig. 8*B*), voC (Fig. 8*C*), and M1 (Fig. 8*D*) are also provided. We estimated the diffusion spread to be ~1 mm in diameter based upon the distribution of active and inactive sites shown in Figure 8*A*.

Intra-vmPFC infusion of δ- or κ-selective opioid agonists did not produce DAMGO-like effects

To test whether stimulation of δ- and/or κ-opioid receptors would produce food intake and motor effects similar to those obtained with μ-receptor stimulation, we infused DPDPE (δ-selective agonist) and LPK-26 (κ-selective agonist) into the vmPFC of separate groups of *ad libitum*-fed rats. Doses of DPDPE and LPK-26 were matched to DAMGO doses based on the K_D (dissociation constant) values of all peptides. Intra-vmPFC infusions of these peptides failed to significantly affect food intake ($F_{(2,14)} = 0.51$, NS; $F_{(2,14)} = 0.37$, NS, respectively, in Fig. 9*A* and *B*). Feeding- and exploratory-related behaviors, including mean eating bout duration, total water intake, mean drinking bout duration, locomotor activity, and total number of rears, are summarized in Table 2. Briefly, DPDPE infusions failed to alter any of these feeding and behavioral microstructure measures, while LPK-26 infusions significantly increased mean eating bout duration ($F_{(2,14)} = 3.79$, $p < 0.05$), concomitantly decreasing mean drinking bout duration ($F_{(2,14)} = 6.06$, $p < 0.05$) and locomotor activity ($F_{(2,14)} = 4.35$, $p < 0.05$). A nonsignificant trend toward a decrease in rearing bouts was also observed ($F_{(2,14)} = 2.89$, NS). Thus, neither δ- nor κ-receptor stimulation reproduced the effects of μ-receptor stimulation; indeed, the profile associated with intra-vmPFC κ-receptor stimulation was essentially the opposite of that seen with DAMGO.

Stimulation or blockade of vmPFC dopamine, serotonin, or norepinephrine systems failed to produce DAMGO-like behavioral effects

To examine the pharmacological specificity of the DAMGO-induced feeding and microstructure effects beyond opioid systems, we manipulated the three main vmPFC monoamine systems by pharmacologically activating or blocking dopamine, norepinephrine, and serotonin receptor subtypes. As shown in Figure 10, *A*, *B*, and *C*, infusions of equimolar concentrations of dopamine-, serotonin-, and norepinephrine-hydrochloride into the vmPFC failed to significantly affect food intake in *ad libitum*-fed animals ($F_{(3,15)} = 0.51$, NS; $F_{(3,15)} = 0.23$, NS; $F_{(3,21)} = 1.72$, NS, respectively). Infusions of SCH23390, raclopride, WAY100635, MDL11939, prazosin, and a mixture of betaxolol, and ICI118991 were infused into vmPFC of separate groups of food-deprived rats. Food intake, mean eating bout duration, water intake, mean drinking bout duration, locomotor activity, and number of rears are summarized in Table 3. SCH23390, WAY100635, and prazosin failed to affect any of the exploratory or ingestive behaviors measured in this study. Infusion of raclopride (2.0 μg/0.5 μl) increased food intake slightly compared with vehicle ($F_{(2,12)} = 5.04$, $p < 0.05$). Intra-vmPFC infusions of MDL11939 led to a very strong trend toward an increase in food intake in the overall ANOVA ($F_{(2,20)} = 3.49$, $p = 0.05$), with an inverted-U-shaped dose-effect function. Preplanned contrasts revealed a significant increase in food intake between vehicle and a low dose of MDL11939 ($p < 0.05$). Preplanned contrasts also revealed a significant increase in mean eating bout duration between vehicle and low MDL11939 dose ($p < 0.05$). Water intake was increased ($F_{(2,20)} = 4.63$, $p < 0.05$) and number of rear bouts was decreased ($F_{(2,20)} = 3.71$, $p < 0.05$) by MDL11939 injection. Finally, intra-vmPFC infusion of betaxolol/ICI118551 mixture significantly decreased locomotor activity ($F_{(1,3)} = 36.91$, $p < 0.01$) while leaving all other measures unaffected. In summary, none of the monoamine agonists or antagonists recapitulated the behavioral profile seen with intra-vmPFC μ-opioid stimulation. Blockade of dopamine receptor D_2 and 5-hydroxytryptamine receptor 2A (5HT2A) produced modest increases in food intake; however, the overall profile of motor activity was dissimilar (or even opposite) to that seen with DAMGO. These experiments reveal the remarkable pharmacological selectivity of the μ-receptor-mediated feeding effect.

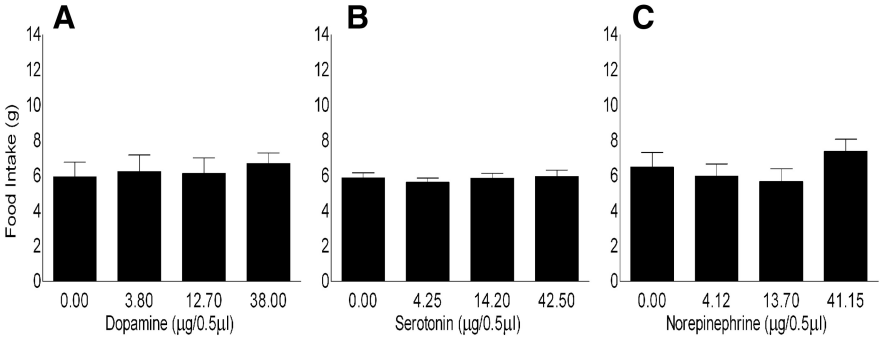

Figure 10. ***A–C***, Effects over a 60 min test session of intra-vmPFC dopamine (***A***), serotonin (***B***), and norepinephrine (***C***) on food intake in food-deprived rats. Values represent mean ± SEM for each treatment. Drug doses are in micrograms per 0.5 μl per side.

Discussion

Summary of results

DAMGO infusions into vmPFC (anterior prelimbic area) and voC, but not dorsal or lateral frontal areas, produced intense feeding responses under *ad libitum* and food-deprived conditions. This feeding effect was accompanied by significant hyper-

Table 3. Ingestive and exploratory behaviors following administration of selective monoamine antagonists into vmPFC

	Food intake (g)	Mean eat bout duration (s)	Water intake (ml)	Mean drink bout duration (s)	Locomotor counts	Rearing counts
Muscimol-induced inactivation						
0	5.2 ± 0.38	25.53 ± 1.98	4.1 ± 0.55	18.46 ± 3.97	51.57 ± 5.35	64.57 ± 8.96
75	5.3 ± 0.25	52.69 ± 3.79**	5.1 ± 0.57	16.12 ± 1.33	38.86 ± 5.93*	42.57
SCH23390						
0	6.1 ± 0.64	60.4 ± 10.56	4.9 ± 0.61	27.2 ± 4.68	24.4 ± 3.93	51.0 ± 8.09
1	6.8 ± 0.51	58.8 ± 9.68	4.4 ± 0.85	25.6 ± 5.11	17.5 ± 2.73	36.6 ± 6.02
2	5.4 ± 0.58	64.1 ± 9.52	4.7 ± 0.60	24.6 ± 3.19	18.9 ± 2.42	37.8 ± 4.44
Raclopride						
0	4.4 ± 0.48	48.3 ± 8.20	4.2 ± 1.0	19.4 ± 2.80	26.4 ± 5.15	53.0 ± 12.79
1	5.1 ± 0.43	47.1 ± 10.41	3.9 ± 0.90	22.6 ± 4.25	26.6 ± 3.88	65.7 ± 12.49
2	5.7 ± 0.45**	59.4 ± 12.97	3.8 ± 0.47	23.6 ± 3.95	21.7 ± 5.28	50.9 ± 10.43
WAY100635						
0	6.2 ± 0.91	52.5 ± 9.57	7.0 ± 0.35	38.9 ± 11.37	24.8 ± 4.37	42.8 ± 5.74
2	5.6 ± 0.50	53.0 ± 8.06	5.1 ± 0.83	24.6 ± 4.40	26.8 ± 5.84	56.0 ± 15.64
MDL11939						
0	4.4 ± 0.62	45.6 ± 14.61	2.6 ± 0.58	18.9 ± 3.04	25.9 ± 3.91	57.5 ± 10.34
0.15	5.8 ± 0.55*	68.1 ± 12.57*	3.5 ± 0.67	26.7 ± 7.16	19.1 ± 2.44	35.4 ± 6.24*
0.45	5.5 ± 0.40	50.2 ± 10.08	4.5 ± 0.46**	24.7 ± 2.94	26.0 ± 2.15	53.5 ± 5.51
Prazosin						
0	4.6 ± 0.42	50.5 ± 9.81	4.6 ± 0.75	26.0 ± 3.97	30.8 ± 4.61	45.3 ± 6.88
0.25	4.4 ± 0.48	56.0 ± 12.61	4.8 ± 0.25	26.3 ± 3.88	28.8 ± 3.35	41.8 ± 10.68
B1 and B2 mixture						
0	5.0 ± 0.29	52.0 ± 10.46	4.4 ± 0.63	20.8 ± 3.86	30.8 ± 2.75	62.3 ± 11.39
1/1	4.6 ± 0.29	53.8 ± 7.81	3.1 ± 0.63	15.4 ± 1.90	25.0 ± 3.34**	50.3 ± 8.38

Values represent mean ± SEM. *$p < 0.05$, **$p < 0.01$, compared with vehicle. All drug doses are in units of micrograms per 0.5 μl, except for muscimol dose, which is in units of nanograms per 0.5 μl.

activity and abrupt switching between ingestive and exploratory behaviors, reducing the average duration of individual behavioral events. These results were reproduced neither by stimulation of ∂- or κ-opioid receptors, dopaminergic, serotonergic, or noradrenergic receptors, nor by blockade of specific monoamine receptor subtypes. DAMGO in the vmPFC elicited neither nonspecific gnawing behaviors nor drinking in thirsty rats, suggesting that the DAMGO-induced effect was specific to feeding. Furthermore, in a carbohydrate versus fat food-choice test, intra-vmPFC DAMGO selectively augmented intake of a palatable carbohydrate-enriched diet even in rats with baseline fat preferences. Together, these findings represent the first identification of neocortical sites supporting opioid-mediated hyperphagia, and the first demonstration that a prefrontal cortical manipulation is sufficient to provoke intake of specific foods even under low-drive conditions.

Pharmacological specificity of μ-opioid-mediated effects

The failure of monoamine drugs, or other opiate agonists, to produce DAMGO-like effects cannot be attributed to the use of insufficient doses; the doses for all compounds are behaviorally active (Knapp et al., 1991; Bakshi and Kelley, 1993; Dhawan et al., 1996; Delfs et al., 2000; Baldo et al., 2002; Carli et al., 2006; Pehek et al., 2006; Tao et al., 2008). All these neuromodulator receptors are present in cortex (Mansour et al., 1987; Briand et al., 2007), and dopamine, serotonin, and norepinephrine efflux in the medial PFC increases during feeding (Bassareo et al., 2002; Ahn and Phillips, 2003; Fallon et al., 2007). Hence, although monoamine release in the PFC is influenced by feeding, and monoamines regulate other PFC functions such as working memory and attention (Robbins and Arnsten, 2009), basic feeding responses cannot be driven by stimulation of these systems alone. It appears that some μ-opioid-induced alteration of PFC network function is required.

Presently, the mechanisms through which opioid receptors modulate PFC network function are unknown. GABAergic interneurons within the prefrontal cortex express μ-opioid receptors (Taki et al., 2000), and μ-opioid receptor stimulation inhibits these interneurons (Witkowski and Szulczyk, 2006). This suggests that stimulation of μ-opioid receptors disinhibits evoked pyramidal cell firing (Férézou et al., 2007), akin to the actions of μ-opioids in the hippocampus (Zieglgänsberger et al., 1979; Madison and Nicoll, 1988; Capogna et al., 1993). Other *in vitro* studies have shown that μ-opioid agonists reduce evoked firing in prefrontal pyramidal cells, via an inhibition of 5HT2A-mediated presynaptic glutamate release (Marek and Aghajanian, 1998; Marek et al., 2001). Hence, there are two opposing explanations for our behavioral effects, as follows: (1) a net increase in PFC outflow due to local disinhibition of pyramidal cells; versus (2) a net decrease in PFC outflow resulting from the attenuation of excitatory input to pyramidal cells.

The first mechanism may be a more likely candidate to explain our results. First, as shown here, muscimol-induced inactivation of vmPFC produces a behavioral profile opposite to that seen with DAMGO (Table 3). In this regard, it is interesting that muscimol-like features were seen after stimulation of κ-opioid receptors or blockade of 5HT2A receptors, suggesting that these two manipulations achieved their behavioral effects via a net decrease in PFC output. Second, based on dissimilarities between the effects of MDL11,939 and DAMGO, it seems unlikely that a DAMGO-induced suppression of 5HT2A-driven glutamate release could entirely explain the present data. Hence, the present results seem consistent with a model in which the net effect of local DAMGO administration is to suppress a local inhibitory component of PFC network function.

Anatomical specificity

Given that μ-opioid receptor distribution is fairly homogeneous in both our positive and negative sites (Mansour et al., 1987), it is likely that the anatomical specificity of our effect is conferred by the input/output connectivity of the behavioral "hotspots." The prelimbic, infralimbic, and ventral orbital cortex project directly

to several feeding-related sites in the ventral forebrain, such as the Acb shell and lateral hypothalamus (LH) (Hurley et al., 1991; Takagishi and Chiba, 1991; Risold et al., 1997; Floyd et al., 2001; Vertes, 2004). Indeed, retrograde tracers in the Acb or LH yield labeling in frontal cortex (Gabbott et al., 2005) that conforms remarkably well to the active sites in the current study. Furthermore, stimulating glutamate receptors within the LH induces hyperactivity and strong feeding responses (Stanley et al., 1996). Hence, if μ-receptor stimulation enhances vmPFC glutamatergic output, as proposed above, the LH may represent an important downstream effector site for the observed behavioral effects.

Behavioral specificity

The DAMGO-induced feeding effect reflects neither (1) nonspecific oromotor effects (chewing/gnawing) nor (2) the general promotion of the response set for which the animal is primed. Hence, when water-deprived animals were given a concurrent choice either to drink water or to eat, intra-vmPFC DAMGO selectively augmented feeding. Similarly, when rats were presented with a choice between carbohydrate- or fat-enriched test diets, intra-vmPFC DAMGO increased carbohydrate intake, even in subjects that exhibited baseline fat-enriched diet preference. These observations strongly suggest that intra-vmPFC DAMGO is not simply augmenting a drive-induced, prepotent, high-frequency behavior or enhancing a response bias stemming from intrinsic preference, but is instead establishing a specific food-directed central motivational state (CMS).

The mechanisms underlying this motivational specificity are unknown. One possibility is that intra-PFC μ-receptor stimulation augments the reward valuation of carbohydrate-enriched foods, akin to what is observed in the Acb for fat-enriched foods (Zhang et al., 2003). This would account for the increased carbohydrate intake in the macronutrient choice experiment. Another interesting possibility is that the intense carbohydrate hyperphagia could represent an attempt to "quell" a stress-like aversive state stemming from DAMGO-induced PFC overactivity. It is well established that the PFC is significantly activated by stress (Deutch and Roth, 1990; Cullinan et al., 1995), resulting in diminished PFC-specific cognitive functions such as working memory (Murphy et al., 1996; Stalnaker et al., 2009). Certain aspects of the DAMGO-induced behavioral profile would tend to support this idea, as follows: (1) DAMGO-treated rats generate copious fecal boli (data not shown), indicative of heightened arousal or stress; and (2) feeding bout duration is shortened, in contrast to sustained eating patterns seen with *ad libitum*-maintained animals given palatable food (Will et al., 2003). In fact, it has been postulated that stress-induced palatable feeding (including intake of carbohydrate-rich foods) stems partly from an attempt to dampen stress effects (Dallman et al., 2003; Ulrich-Lai et al., 2007). Last, it should be noted that, at present, it is impossible to determine whether the selective stimulation of carbohydrate intake was related to changes in post-ingestive macronutrient detection or in orosensory perception. Further work is needed to distinguish among these possible mechanisms.

Another prominent feature of the DAMGO-induced behavioral profile was the qualitative "fragmentation" of the motor repertoire. The observed rapid shifting among different motor responses is not inconsistent with what one might expect from the overstimulation of a site that plays an executive role in preventing response perseveration (Ragozzino et al., 1999; Passetti et al., 2002). Interestingly, a recent meta-analysis of clinical studies has suggested that executive dysfunction in attention-deficit/hyperactivity disorder (ADHD) is associated with obesity (Cortese et al., 2008). Indeed, the fragmented motor profile may have face validity for the behavioral symptoms seen in ADHD. The implications from such a relationship could be profound, as it may reveal a previously unknown mechanism by which disruptions in cortical network activity could produce dysregulated feeding responses and contribute to metabolic/obesity phenotypes in several illnesses that are associated with PFC dysfunction. Clearly, additional work is needed to refine our understanding of the behavioral functions of frontal cortical μ-opioids. Nevertheless, the present results indicate that μ-opioid stimulation in frontal cortical regions induces a specific food-directed CMS, along with producing several behavioral changes consistent with a high-arousal, stress-like state.

Conclusions

To our knowledge, this is the first demonstration that local manipulations of intra-PFC μ-opioid systems can provoke feeding behavior and promote carbohydrate-enriched diet selection. These results may be important for understanding the etiology of eating disorders, in which changes in affect can alter feeding behavior in a manner often strongly incompatible with homeostatic needs (Lowe and Fisher, 1983; Fitzgibbon et al., 1993; Ahlberg et al., 2002). Several studies have provided convergent evidence that medial prefrontal and orbitofrontal regions exhibit aberrant patterns of activation in individuals with eating disorders (Uher et al., 2003, 2004; Felsted et al., 2010); however, the role of these frontal regions in the etiology of dysregulated feeding responses is unclear. It is generally understood that frontal cortex exerts a general "supervisory" function over motivational states that are generated at earlier levels of processing (Berthoud, 2004; Petrovich and Gallagher, 2007; Robbins, 2007). However, the present data indicate that the stimulation of the μ-opioid system in restricted zones of frontal cortex is sufficient to instantiate a behaviorally specific feeding drive, with features suggesting linkage to a PFC-generated high-arousal state. These findings may provide a neuropharmacological and systems framework for understanding how heightened arousal or stress can, in certain instances, elicit appetitive behaviors in the context of eating disorders and/or other disorders of frontal executive function.

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