

ARTICLE



Dissociable control of μ -opioid-driven hyperphagia vs. food impulsivity across subregions of medial prefrontal, orbitofrontal, and insular cortex

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This study explored potentially dissociable functions of mu-opioid receptor (μ -OR) signaling across different cortical territories in the control of anticipatory activity directed toward palatable food, consumption, and impulsive food-seeking behavior in male rats. The μ -OR agonist, DAMGO ([D-Ala², N-Me-Phe⁴, Gly⁵-ol]-enkephalin), was infused into infralimbic cortex (ILC), prelimbic cortex (PrL), medial and lateral ventral orbitofrontal cortices (VMO, VLO), and agranular/dysgranular insular (AI/DI) cortex of rats. Intra-ILC DAMGO markedly enhanced contact with a see-through screen behind which sucrose pellets were sequestered; in addition, rats having received intra-ILC and intra-VMO DAMGO exhibited locomotor hyperactivity while the screen was in place. Upon screen removal, intra-ILC and -VMO-treated rats emitted numerous, brief sucrose-intake bouts (yielding increased overall intake) interspersed with significant hyperactivity. In contrast, intra-AI/DI-treated rats consumed large amounts of sucrose in long, uninterrupted bouts with no anticipatory hyperactivity pre-screen removal. Intra-PrL and intra-VLO DAMGO altered neither pre-screen behavior nor sucrose intake. Finally, all rats were tested in a sucrose-reinforced differential reinforcement of low rates (DRL) task, which assesses the ability to advantageously withhold premature responses. DAMGO affected (impaired) DRL performance when infused into ILC only. These site-based dissociations reveal differential control of μ -OR-modulated appetitive/approach vs. consummatory behaviors by ventromedial/orbitofrontal and insular networks, respectively, and suggest a unique role of ILC μ -ORs in modulating inhibitory control.

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INTRODUCTION

The motivation to seek and consume food is essential for survival. However, when food motivation is unchecked by normal inhibitory controls, goal-seeking responses become excessive or intrusive, and consumption surpasses homeostatic requirements. A better understanding of the anatomical networks and neurochemical systems that initiate and adaptively limit natural appetitive behaviors, such as feeding, may uncover principles that apply to a wide variety of addictions, eating disorders (EDs), and other conditions with common underlying features of motivational dysfunction.

Decades of research into the central control of feeding behavior has established that mu-opioid receptor (μ -OR) signaling in discrete subcortical loci (including the nucleus accumbens [1], central amygdala [2], and hypothalamus [3]) is sufficient to drive intense feeding responses, including for palatable or preferred foods [4–6]. The feeding-modulatory role of cortically localized μ -ORs, however, has been comparatively understudied. Nevertheless, recent work has begun to identify cortical loci, in which μ -OR stimulation produces motivational effects rivaling those seen in extensively studied subcortical sites, such as the nucleus accumbens. For example, μ -OR stimulation in a circumscribed area

of ventromedial prefrontal cortex (vmPFC), alone among a variety of monoamine or amino acid neurotransmitter manipulations, augments the intake of both standard and palatable food, selectively enhances feeding in a concurrent food/water choice in fluid-restricted rats, and robustly increases the willingness to work for sucrose reward in a progressive-ratio task, even in rats with low hunger drive [7–9]. Furthermore, intra-vmPFC μ -OR signaling, but not monoamine stimulation, produces a marked impulsivity-like impairment of inhibitory control in a sucrose-reinforced differential reinforcement of low rates (DRL) task, which assesses the ability to withhold disadvantageous, prepotent food-seeking responses [9]. To date, no other neurochemical manipulation of the vmPFC has been found to produce this behavioral profile. Hence, μ -OR stimulation may represent a unique tool with which to “push” cortical sites to reveal feeding-related functions. Accordingly, recent studies have begun to identify additional loci beyond vmPFC, in rat ventral orbitofrontal and posterior insular cortex, where μ -OR stimulation enhances feeding and related functions, such as hedonic taste reactivity [10]. The clinical relevance of these observations is bolstered by the growing number of human ligand-PET and fMRI neuroimaging studies showing μ -OR changes within homologous medial, orbitofrontal,

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and insular cortical regions in conditions pertinent to appetitive motivation and its dysregulation, such as feeding and viewing food images [11, 12], obesity and EDs [13–15], various stages of the drug addiction cycle [16–19], and impulsivity [20].

To better understand the functions of cortical μ -OR systems, the present study mapped frontal-cortical μ -OR agonist actions on dissociable components of palatable food-motivated behavior: behavior antecedent to expected food access, actual consumption, and inhibitory control over food-seeking behavior. Our overarching goal was to determine the extent to which these functions are either overlapping or segregated to distinct nodes in frontal-cortical circuitry. Small-volume infusions of the selective μ -OR agonist, DAMGO ([D-Ala², N-Me-Phe⁴, Gly⁵-ol]-enkephalin), were placed in rat vmPFC or dorsomedial prefrontal cortex (infralimbic cortex (ILC) and prelimbic cortex (PrL) regions, respectively), orbitofrontal cortex (ventromedial (VMO) and lateral orbitofrontal regions (VLO)), or insular cortex (agranular/dysgranular regions at the level of primary gustatory cortex (AI/DI)). All these sites have appreciable densities of μ -ORs, with no evidence for major differences in receptor expression across sites [21, 22]. Using a within-subjects design, rats were tested in two paradigms: an observational test in which spontaneous behaviors prior to expected sucrose access and during access were recorded and scored; and the abovementioned DRL task of inhibitory control. Of particular interest was determining the degree to which cortical sites subserving μ -OR-mediated effects on inhibitory control anatomically overlap the sites that generate anticipatory activity, consumption, or both.

METHODS

Subjects

Subjects were male Sprague–Dawley rats (Harlan, Madison, WI) weighing 275–300 g upon arrival at the laboratory. Rats were housed in a light- and temperature-controlled vivarium, under a 12:12 h light–dark cycle (lights on at 0700 h). Food (Envigo Teklad laboratory diet) and water were available ad libitum, except as indicated for specific experiments. Animals were handled daily to reduce stress. Testing occurred between 1100 and 1500 h. All facilities and procedures were in accordance with NIH guidelines and were approved by the Institutional Animal Care and Use Committee of Univ. Wisconsin–Madison.

Differential reinforcement of low response rates (DRL-15 s)

Training and testing procedures were conducted in standard operant chambers, as described previously [9].

All rats underwent an initial training period during which they were maintained at $90 \pm 2\%$ of free-feeding body weight using scheduled feedings. During this phase, rats were trained to lever press on a conjoint random-time 30 s/fixated ratio 1 schedule (RT-30 s/FR-1) and then a FR-1 schedule [9] to earn sucrose pellets (BioServe Dustless Precision Sucrose Pellets, 45 mg; Product # F0023). Once consistent responding was achieved on FR-1, rats advanced to a variable-interval 15 s schedule (VI-15 s), VI-30 s schedule, and finally to a DRL-15 s schedule. In the DRL-15 s schedule, rats were required to withhold responding after reinforcer delivery during an unsignaled, fixed time period (15 s). After this delay, subjects could respond to earn the next reinforcer. However, each premature response (i.e., one that was not separated from the previous response by at least 15 s) reset the delay timer. For optimal performance, therefore, the timing of consecutive responses (inter-response intervals) must exceed the delay interval. After 2–3 days on DRL-15 s, rats were returned to ad libitum feeding, with food withdrawn in the 2 h immediately preceding each testing session. Rats were then tested under this new food restriction schedule until stable baseline responding was achieved ($\pm 15\%$ variability or less) in reinforced responses over three consecutive testing days. DRL sessions lasted ~ 20 min.

Behavior-observation procedure

Rats were tested in clear polycarbonate cages (9.5 in width \times 17 in length \times 8 in height), identical to their home cages except for wire grid floors. Pre-measured amounts of sucrose pellets (the same type of sucrose

pellets that were used in the DRL procedure described above) were placed in clear glass jars affixed to the wire grid. For the first part of the session, chicken-wire screens blocked access to the sucrose, while allowing it to be seen. All sessions started with screens in place; screens were removed after 15 min to allow 45 min of access to and consumption of the sucrose pellets. Rats were videotaped with a digital camcorder for the entire 60 min session (i.e., 15 min with screens in place, 45 min after screen removal). After the conclusion of testing, an experimenter blind to treatment viewed the digital files, and recorded number and length of bouts of screen contacts, spontaneous ambulation (cage crossings), rearing, and eating, using an event recorder interfaced to a PC-based desktop computer. Several derivative measures were calculated from these data: mean eating bout duration (total # of bouts/total duration), global eating rate (total intake/total duration), and global eating efficiency (total intake/total # of bouts). To record the duration of a particular behavioral event, a cumulative timer (specific for that behavior) on the event recorder was started at the initiation of the behavior. The timer was switched off when the behavior was interrupted by a different behavior.

Surgical procedures

Stereotaxic surgery under isoflurane anesthesia was carried out, as described previously [23]. Stainless-steel guide cannulae (10-mm-long; 25-gauge) were implanted at flat skull aiming at ILC ($N = 7$ rats; AP +3.0, ML ± 2.25 , DV -5.2 , at 19° from vertical from skull surface), PrL ($N = 8$ rats; AP +3.0, ML ± 2.0 , DV -5.15 , at 23° from vertical, from skull surface), VMO cortex ($N = 9$ rats; AP +3.25, ML ± 1.7 , DV -5.15 , from skull surface), VLO cortex ($N = 7$ rats; AP +3.2, ML ± 2.8 , DV -5.15 , from skull surface), and AI/DI cortex ($N = 7$ rats; AP +1.3, ML ± 5.05 , DV -6.65 , from skull surface). All guide cannulae were implanted 2.5 mm above the final infusion site except for PrL, which is very dorsal, where injectors extended 1 mm beyond the guide cannulae.

Microinfusion procedures and drugs

Intracerebral microinjections were carried out, as previously described [23]. DAMGO was obtained from Bachem. All drugs were dissolved in sterile 0.9% saline.

Experimental design

After a week of recovery from surgery, rats were separated into two groups tested either first in DRL-15 s followed by the behavior-observation procedure, or vice versa, in a counterbalanced block design. All rats were first acclimated to microinjection procedures with sham infusions and intra-site saline injections on consecutive days. Drug testing in either DRL or the behavior-observation procedure commenced 2 days after acclimation. Rats received intracranial infusions of either vehicle (0.9% saline) or DAMGO (0.1, 0.3, or 1.0 μ g). The microinfusion volume was 0.25 μ l/side. Each rat received all those treatments counterbalanced according to latin square designs within each block. Testing began 15 min after microinfusions. At least 1 day was allowed between treatments to allow for drug washout. At the conclusion of dose-response testing, the blocks were reversed, and dose-response testing commenced again.

Histology

At the end of each experiment, rats were deeply anesthetized and perfused with 10% formalin in phosphate buffer. Brains were stored in 10% formalin. Coronal sections (60 μ m) were cut through the infusion site on a cryostat microtome, collected on slides, stained with cresyl violet, and subsequently reviewed to verify correct injection placements.

Statistical analyses

Data were analyzed using repeated measures ANOVAs. Contingent upon significance in the ANOVAs, post hoc comparisons among means were conducted using Tukey's HSD test. The level of statistical significance was set at $P < 0.05$ for all experiments.

RESULTS

Histological analyses

Chartings in Fig. 1A–E depict the placement of injector tips in each of the targeted cortical sites for rats included in the study. Bilateral placements within each rat were never separated from one another by > 0.4 mm in the anteroposterior plane. The

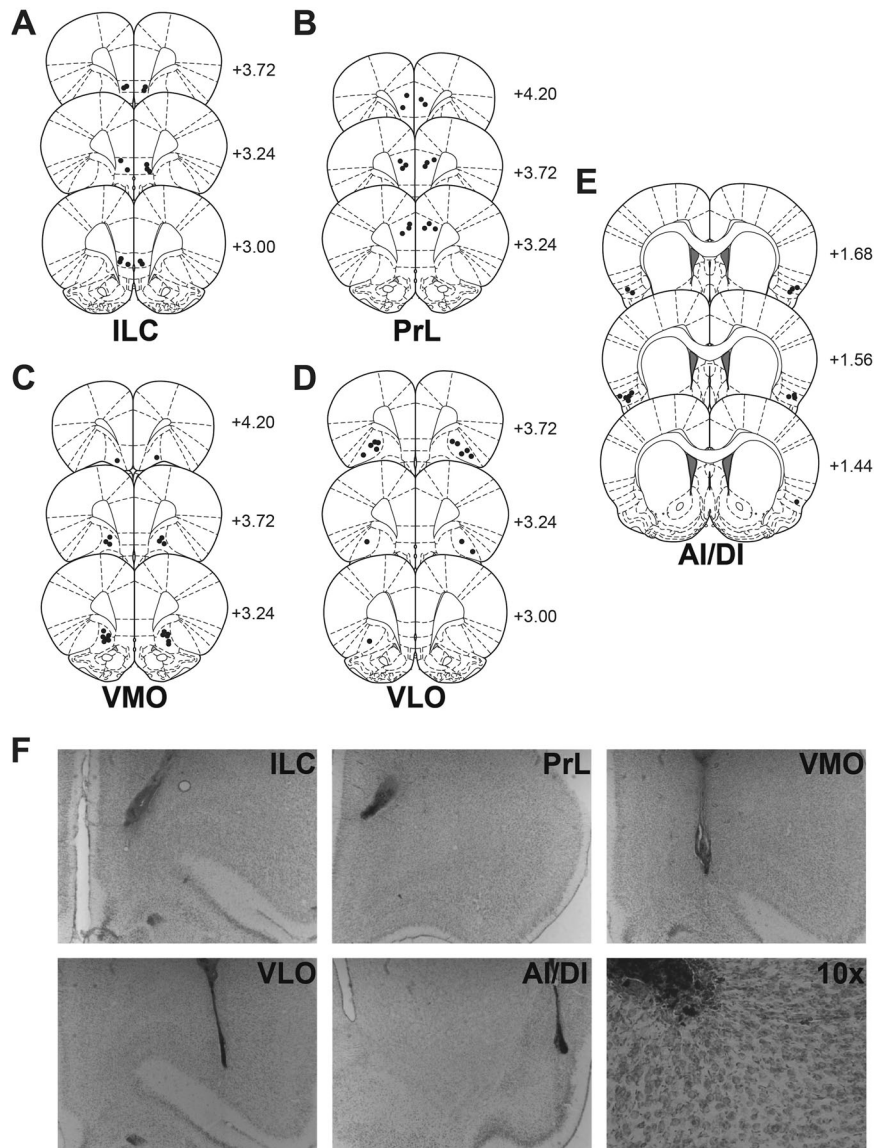


Fig. 1 Chartings depicting bilateral microinjector tip placements for all experiments followed by demonstrative histology for each site. **A** ILC infralimbic cortex ($N=7$). **B** PrL prelimbic cortex ($N=8$). **C** VMO ventromedial orbitofrontal cortex ($N=9$). **D** VLO ventrolateral orbitofrontal cortex ($N=7$). **E** AI/DI agranular/dysgranular insular cortex ($N=7$). Numbers indicate distance from bregma in millimeters. **F** High-power view of an injector tip, showing no unusual damage and good cell integrity at the injection site.

photomicrographs in Fig. 1F shows a representative high-power view of injector tip placements in Nissl (cresyl violet)-stained tissue. For included rats, no atypical or excessive damage was noted; examination of the area even closely adjacent to the injector tips revealed intact cells and good tissue integrity (see brightfield photomicrographs in Fig. 1F).

Order of participation in DRL vs. the behavioral-observation procedure had no effect

All rats were subjected to a within-subject counterbalanced design, in which they were tested in either a behavioral-observation testing block first, and operant DRL-15 s testing block second, or vice versa (see “Methods”). No effects of block order were detected for any site or measure ($F_s = 0.06$ – 2.54 , not significant (NS)).

Intra-ILC and intra-VMO DAMGO-modulated behavior prior to contact with food

Dissociable effects of DAMGO were noted across cortical sites in the first 15 min of the behavioral-observation test, while the wire-

mesh screen preventing food contact was still in place. Intra-ILC DAMGO increased the number of screen contacts ($F(3,18) = 3.22$, $P = 0.047$; Fig. 2A), which consisted of sniffing and contacting the screen with the paws (i.e., treating the screen as a barrier), but with no licking, gnawing, or other obvious ingestive-behavior fragments. No statistically significant effects of DAMGO on screen contacts were detected for any of the other sites ($F_s = 0.06$ – 2.30 , NS). In addition, DAMGO increased the number of cage crossings while the screen was in place for ILC ($F(3,18) = 8.05$, $P = 0.001$) and VMO ($F(3,24) = 5.58$, $P = 0.005$), but not for PrL, VLO, or AI/DI ($F_s = 0.26$ – 2.11 ; NS, Fig. 2B).

Intra-site DAMGO infusions augmented sucrose-pellet intake in ILC, VMO, and AI/DI

In the 45-min period after the screen was removed from the sugar pellets, DAMGO microinfusions significantly increased sucrose intake in three of the five sites tested (see Fig. 3). For sub-territories of medial PFC, DAMGO infusions into ILC elicited significant sucrose hyperphagia ($F(3,18) = 8.31$, $P = 0.001$), while

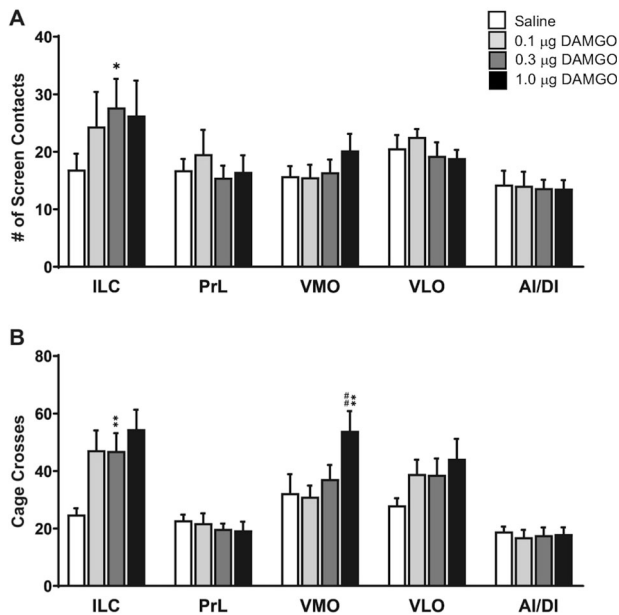


Fig. 2 DAMGO-induced effects in the behavior-observation procedure, in the first 15 min of the test during which a screen obstructed access to sucrose pellets. A Screen contacts and **B** locomotor activity are shown. * Different than saline ($P < 0.05$), ** different than saline ($P < 0.01$), ## different than 0.1 µg DAMGO ($P < 0.01$). Error bars depict 1 SEM. ILC infralimbic cortex, PrL prelimbic cortex, VMO ventromedial orbitofrontal cortex, VLO ventrolateral orbitofrontal cortex, AI/DI agranular/dysgranular insular cortex.

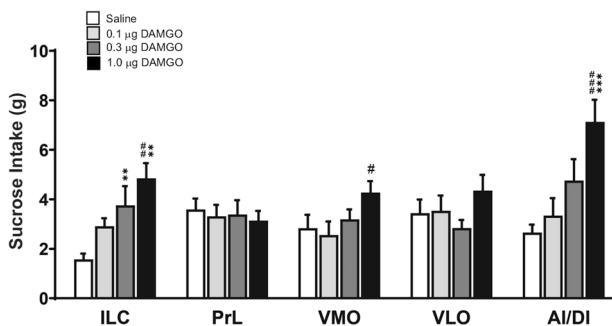


Fig. 3 Effects of DAMGO treatment on sucrose-pellet intake in the 45-min session after obstructing screens were removed from the pellet jars (see “Methods”). ** $P < 0.01$, *** $P < 0.001$, different from saline. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ different than 0.1 µg DAMGO. Error bars depict 1 SEM. ILC infralimbic cortex, PrL prelimbic cortex, VMO ventromedial orbitofrontal cortex, VLO ventrolateral orbitofrontal cortex, AI/DI agranular/dysgranular insular cortex.

no effects were noted more dorsally in PrL ($F(3,21) = 0.38$, NS). In orbitofrontal cortex, a DAMGO-induced increase in sucrose intake was observed in VMO ($F(3,24) = 3.03$, $P = 0.049$), but not more laterally in VLO ($F(3,18) = 1.79$, NS). The magnitude of DAMGO's effect on intake was similar in ILC and VMO, averaging 4.8 and 4.3 g of sucrose, respectively, after the 1.0 µg dose. Intra-AI/DI DAMGO infusions also elicited considerable sucrose hyperphagia ($F(3,18) = 9.08$, $P = 0.0007$), with intake at the 1.0 µg dose averaging 7.1 g.

DAMGO provoked dissociable feeding-behavior patterns across cortical sites

In the 45-min post-screen testing period, intra-ILC DAMGO-induced augmentation of sucrose intake was characterized by a

significantly higher number of eating bouts initiated ($F(3,18) = 3.65$, $P = 0.032$; Fig. 4A), a significant but small increase in total eating duration ($F(3,18) = 3.77$, $P = 0.029$; Fig. 4B). These changes resulted in a significant reduction in mean eating bout duration ($F(3,18) = 7.14$, $P = 0.0023$), and corresponding reduction in gram intake per bout ($F(3,18) = 5.20$, $P = 0.009$); however, global eating rate (gram intake/min) was unchanged. These findings are summarized in Supplementary Materials, Table S1. Also shown in Fig. 4B, DAMGO increased the number of eating bouts when infused into VMO ($F(3,24) = 6.83$, $P = 0.002$) or VLO ($F(3,18) = 3.79$, $P = 0.029$); however, effects in these sites on eating duration trended upward slightly but did not achieve statistical significance ($F_s = 0.91$ – 2.38 , NS; Fig. 4B). Similarly to ILC, mean eating bout duration was significantly decreased in VMO ($F(3,24) = 8.09$, $P = 0.0007$; Supplementary Materials, Table S1). Moreover, global eating rate (gram intake per minute) was significantly increased in VMO alone among all sites ($F(3,24) = 3.44$, $P = 0.033$; Supplementary Materials, Table S1). Together, these results indicate that feeding responses elicited from ILC and VMO were intense, but very brief and fragmentary. The opposite pattern was seen in AI/DI, where DAMGO had a relatively small but still significant effect on number of eating bouts ($F(3,18) = 3.38$, $P = 0.041$), while more than doubling total eating duration ($F(3,18) = 8.56$, $P = 0.001$; Fig. 4A, B). Mean eating bout duration, gram intake per bout, and global eating rate also were dose-relatedly increased in AI/DI, although these effects were not statistically significant ($F_s = 1.58$ – 1.67 , NS; Supplementary Materials, Table S1). Finally, in PrL, DAMGO failed to alter eating bouts, duration, global eating rate or efficiency ($F_s = 0.17$ – 1.35 , NS), but produced a small but significant decrease in mean bout duration at the highest dose ($F(3,21) = 3.81$, $P = 0.025$); these findings are shown in Fig. 4 and in Supplementary Materials, Table S1.

Overall, the same behavioral patterns and site dissociations described above for the entire 45-min post-screen session had begun to appear as early as the first 10 min of the 45-min post-screen periods (see Fig. 4 insets). Thus, DAMGO increased the number of eating bouts when infused into ILC ($F(3,18) = 4.65$, $P = 0.001$) and VMO ($F(3,24) = 8.83$, $P = 0.0004$). These effects on bout initiation did not translate into statistically significant increases in overall eating duration ($F_s = 0.47$ – 1.23 , NS), indicating that the feeding bouts were very short and fragmentary. This conclusion was further bolstered by the finding that mean bout duration in the ILC was significantly decreased ($F(3,18) = 5.13$, $P = 0.0097$; Supplementary Materials, Table S1). In contrast, DAMGO infusions into AI/DI did not increase the number of eating bouts initiated in the first 10 min of the session ($F(3,18) = 0.67$, NS), but greatly increased feeding duration ($F(3,18) = 5.99$, $P = 0.005$). These analyses confirm the qualitative observation that, after screen removal, intra-AIC DAMGO-treated rats moved directly into the sugar jars and consumed the pellets in long, uninterrupted bouts, in strong contrast to the “fragmented” behavioral pattern observed after DAMGO infusions into ILC or VMO.

DAMGO microinfusions elicited motor hyperactivity in the presence of food

In the 45-min post-screen period, DAMGO elicited hyperactivity, indexed by cage crossings, in ILC ($F(3,18) = 8.01$, $P = 0.001$), VMO ($F(3,24) = 5.90$, $P = 0.004$), VLO ($F(3,18) = 7.36$, $P = 0.002$), and PrL ($F(3, 21) = 3.74$, $P = 0.003$); all shown in Supplementary Materials, Fig. S1. In contrast, infusions of DAMGO into the AI did not alter total cage crossings, ($F(3,18) = 1.26$, NS) despite strong modulation of feeding at the same doses. To adjust for the different overall feeding durations obtained in different sites, we calculated the number of cage crossings per unit time the animals were not eating (i.e., cage crossings/[total session duration – total eating duration]), which provided an index of locomotion rate. This adjustment revealed significant dose-related increases in locomotion rates across sites (ILC: $F(3,18) = 6.39$, $P = 0.0039$; VMO:

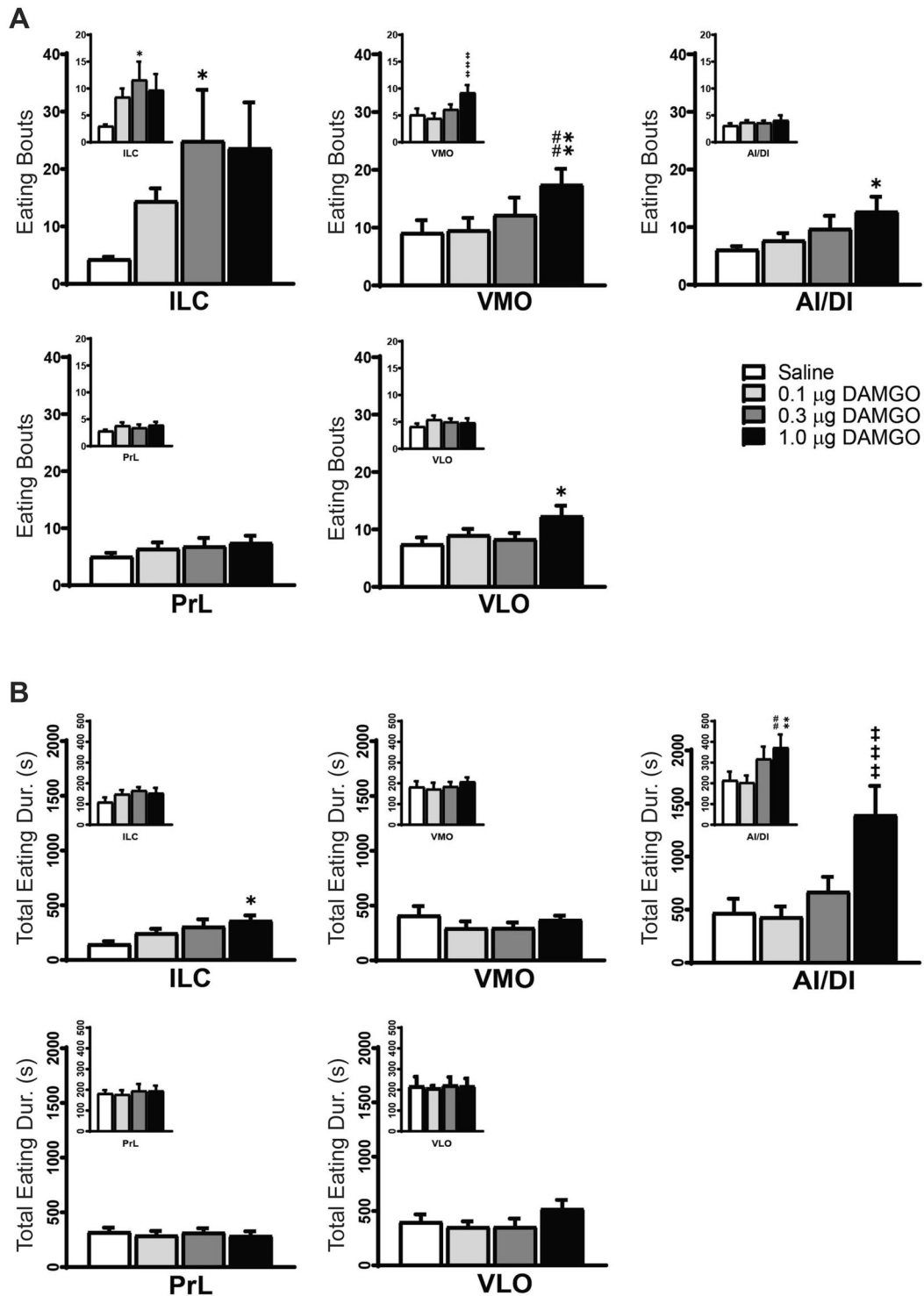


Fig. 4 The effects of DAMGO on eating bouts and eating duration in the 45-min testing session after obstructing screens were removed from the sucrose-pellet jars (see “Methods”). Main figures in **A** and **B** depict eating bouts and eating duration, respectively, for the entire 45-min post-screen testing sessions, while **Insets** depict the first 10 min of the sessions. *, ** Different than saline $P < 0.05$ and $P < 0.01$, ## different than 0.1 µg DAMGO $P < 0.01$, ### different than all other treatments $P \leq 0.001$. Error bars depict 1 SEM. ILC infralimbic cortex, PrL prelimbic cortex, VMO ventromedial orbitofrontal cortex, VLO ventrolateral orbitofrontal cortex, AI/DI agranular/dysgranular insular cortex.

$F(3,24) = 4.94, P = 0.0078$; VLO: $F(3,18) = 7.72, P = 0.0016$, PrL: $F(3,21) = 3.98, P = 0.022$, AI/DI: $F(3,18) = 6.03, P = 0.005$). The rank order of highest mean locomotion rate across sites (crossings/min) was ILC (3.36) > VMO (2.52) > VLO (1.71) > AI/DI (1.29) > PrL (1.09). Hence, the largest increases in locomotion (both total locomotion and locomotion rate during nonfeeding time) were seen in ILC

and VMO. These results are shown in Supplementary Materials, Table S1.

Except in PrL and AI ($F_s = 0.28$ – 1.30 , NS), DAMGO significantly increased general activity in the first 10 min of the 45-min post-screen sessions ($F_s = 3.28$ – $6.55, P_s = 0.004$ – 0.038), as shown in the Supplementary Materials, Fig. S1.

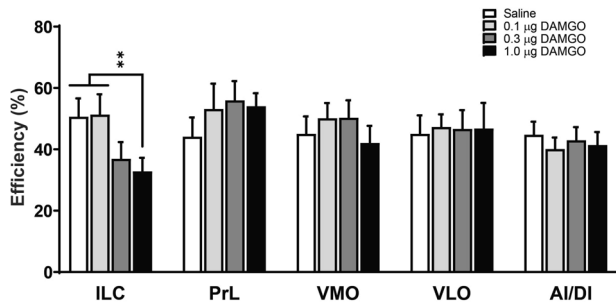


Fig. 5 Effect of DAMGO on DRL performance, presented as response efficiency (reinforced lever presses expressed as a percent of total lever presses). An increase in disadvantageous, premature responding results in a reduction of response efficiency (see “Methods” for further details). $**P < 0.01$. Error bars depict 1 SEM. ILC infralimbic cortex, PrL prelimbic cortex, VMO ventromedial orbitofrontal cortex, VLO ventrolateral orbitofrontal cortex, AI/DI agranular/dysgranular insular cortex.

Intra-site DAMGO impaired DRL-15 performance in ILC only

Performance in the DRL-15 task was indexed by analyzing premature responses (disadvantageous responses emitted during the 15-s waiting period), nose pokes into the sucrose-pellet hopper (an index of generalized motivational and motoric activation), and response efficiency (the proportion of total responses that were successfully reinforced (shown in Fig. 5)). As responding becomes impulsive-like, premature responding increases and efficiency correspondingly decreases. DAMGO dose-relatedly decreased efficiency and increased premature responding only when infused into ILC ($F(6,18) = 5.57$, $P = 0.007$ for response efficiency; $F(6,18) = 3.92$, $P = 0.026$ for premature responses). No DAMGO-induced effects on either of those two measures were noted in any other site ($F_s = 0.10$ – 2.84 , NS). Furthermore, there were no significant effects on nose pokes in any site ($F_s = 0.81$ – 2.60 , NS). Premature responses and nose pokes are summarized in Supplementary Materials, Table S2.

DISCUSSION

The present study revealed marked heterogeneity in the behavioral profiles elicited by μ -OR stimulation across the cortical sites tested. Infusions of DAMGO into ILC and VMO, but not VLO, PrL, or AI/DI, provoked considerable hyperactivity prior to feeding, when a sucrose-filled jar was covered by a see-through mesh screen (and rats had previously learned to expect eventual screen removal). Intra-ILC DAMGO also increased the number of times rats contacted the screen. After screen removal, intra-ILC, intra-VMO, and intra-AI/DI DAMGO-treated rats exhibited sucrose hyperphagia; however, microanalysis of feeding behavior revealed markedly dissimilar behavioral patterns across those sites. Whereas intra-ILC and intra-VMO DAMGO infusions produced numerous, brief, feeding bouts interspersed with frequent bouts of intense hyperactivity, intra-AI/DI DAMGO elicited long, uninterrupted feeding bouts with no hyperactivity before screen removal. In contrast, intra-VLO and intra-PrL DAMGO provoked a modest increase in locomotor activity (only in the presence of food), but no changes in feeding. Finally, DAMGO infusion into ILC but no other site impaired DRL responding by provoking premature-response errors. These results reveal functional specializations across frontal territories in μ -OR modulation of activity and approach, intake, and inhibitory control over food-seeking responses.

Prior intra-cortical DAMGO infusion studies support the present findings of DAMGO-responsive zones in medial prefrontal, orbital, and insular cortices, albeit with some interesting differences. A more limited mapping from our prior work showed sites of

DAMGO-driven food intake clustered around the ILC/PrL boundary and VMO, but not control sites in motor cortex [7]. A more recent study reported that DAMGO augmented both intake and hedonic taste reactivity in medial and lateral orbitofrontal cortex; many of these placements were in the general vicinity of the VMO site probed here, but slightly more anterior [10]. These investigators also report some strong DAMGO-induced feeding responses clustered in the medial wall of PFC, ~ 0.5 – 1.0 mm anterior to the current ILC site (see Fig. 7 in ref. [10]). However, it is unclear whether these investigators probed the specific ILC site tested here. Finally, in insular cortex, sharp anteroposterior gradients of DAMGO-induced intake, hedonic enhancement, and hedonic suppression were found, in which placements ~ 2.0 – 3.0 mm posterior to the site probed here strongly augmented hedonic taste reactivity while producing little effect on intake, while more anterior placements (including the level probed here, where we obtained pronounced hyperphagia) yielded some inconsistent increases in food intake and, interestingly, hedonic suppression. Together, these findings converge on a ventrally localized DAMGO-responsive corridor comprising medial sectors of orbitofrontal and anterior sectors of vmPFC, with gradients of increasing sensitivity as placements move rostrally and ventrally in medial prefrontal cortex, and rostrally and medially in orbitofrontal cortex. Regarding insular cortex, there is evidence that μ -OR stimulation amplifies food intake; however, more work is needed to resolve the anatomical boundaries of this effect more precisely.

Among the most striking of the behavioral dissociations observed in the present study was the difference in the patterns elicited from ILC and VMO vs. AI/DI. Overall, the present results suggest that μ -OR signaling in ILC and VMO (but less so in VLO, and not at all in PrL) is sufficient to generate significant hyperactivity and a food-seeking motivational state, in general agreement with prior findings that intra-ILC DAMGO amplifies effortful sucrose-seeking operant behavior [8, 9] and that medial PFC is strongly activated by food-directed exploratory activity and less so (although still significantly) during consummatory repertoires [24]. Furthermore, the present data bolster prior observations that the overall behavioral phenotype observed after DAMGO stimulation of ventromedial prefrontal and medial orbitofrontal territories is disorganized and fragmented, with bouts of exploratory-like behaviors interrupting bouts of sucrose intake [7]. Nevertheless, despite the similar “fragmented” behavioral patterns elicited from ILC and VMO, impairment of DRL responding was observed only after ILC infusions. In fact, ILC was the only site where DAMGO infusions altered DRL performance; infusions just 1.0 mm dorsal (PrL) or 1.0 mm medial (VMO) did not significantly alter responding, highlighting the circumscribed nature of the effect. Combining results from all the current activity/feeding and DRL experiments reveals the functional specificity of the DRL impairment; neither DAMGO-induced sucrose hyperphagia, nor the generation of hyperactivity either in anticipation of or during sucrose access, represented conditions sufficient to alter DRL responding. Hence, the ILC-localized DRL impairment was not an artifact of generalized arousal or motor activation, nor the obligatory outcome of enhanced feeding motivation. Conversely, when ILC was unperturbed, DRL response efficiency was preserved even in the face of the amplified food motivation and/or hyperactivity variously elicited from the other sites. Studies employing the five-choice serial reaction time task (5-CSRTT) have yielded closely comparable results (like the DRL deficits shown here, premature responding in the 5-CSRTT is considered an index of “waiting” or “action” impulsivity [25]). Lesions of ILC but not of other frontal sites, or glutamatergic or GABA-ergic manipulations of the IL (none of which overtly enhance appetitive motivation), engender marked premature responding in the 5-CSRTT [26, 27]. More generally, it has been hypothesized that ILC contributes to response selection by restraining prepotent or contextually disadvantageous learned

responses, including in extinction [25, 28–31]. Prior neuropharmacological studies of this inhibitory-control function have focused on dopamine, serotonin, and acetylcholine systems [25]. The present results additionally suggest that supernormal μ -OR signaling in the ILC also plays a role, while simultaneously engaging a motivational function (general motivational arousal, “seeking-like” behavior, and disorganized feeding responses) that is more widely distributed through vmPFC plus parts of orbitofrontal cortex, but which is not in itself sufficient to impair inhibitory control in the DRL task.

In contrast to ILC and VMO, μ -OR stimulation in AI/DI affected behavior only during sucrose access, and the ingestive pattern consisted of long, uninterrupted bouts of intake. Considering that the level of insular cortex targeted here corresponds to primary gustatory cortex [32–35], one explanation for this behavioral pattern is that DAMGO enhanced the sensory perception and/or the hedonic evaluation of sucrose actively being tasted. Enhancement of either sensory processing or palatability perception could prolong bouts of contact and ingestion, which is one effect of increasing the concentration and presumably the reward value of sugar [36]. Accordingly, electrophysiological recording studies have identified units and ensembles at a level of insular cortex corresponding to the present infusions that encode chemosensory tasteant characteristics and/or palatability [37–40]. Relatedly, prior work from our group showed that GABA-mediated inactivation of this general area significantly decreased intake of sweet liquid food but not of standard chow pellets [41]. The contrasting behavioral functions of AI/DI-localized μ -ORs and GABA receptors (i.e., hyper- and hypophagia, respectively) could be due to the fact that μ -ORs negatively modulate cortical inhibitory interneurons, thereby disinhibiting local network function [42–45]) in a manner similar to well-established μ -OR actions in hippocampus [46–48]. In contrast, intra-cortical GABA receptor stimulation causes global inhibition of local cortical cellular networks [49, 50].

Units in gustatory cortex also respond to stimuli antecedent to feeding, including food-predictive Pavlovian cues [51], and there is considerable evidence from human neuroimaging studies that insular cortex responds to food images and other food-associated second-order stimuli [52–54]. In this context, the absence of intra-AI/DI DAMGO effects on hyperactivity during anticipation of feeding is noteworthy, particularly given the extensive one- and two-stage connections with the orbitofrontal and medial prefrontal areas that generated anticipatory activity in the present study [33]. The mechanism underlying this behavioral specificity in AI/DI is unclear, but it implies a selective action of μ -ORs on local ensemble dynamics specific to ongoing consummatory activity, specialized μ -OR modulation of output pathways mediating consummatory action patterns, or a combination of the two. μ -OR signaling could also modify a “supervisory” function, such as switching out of eating bouts once they have begun. These effects could modify the contribution of AI/DI to response-selection networks, with the net effect of focusing overall behavioral output on consumption. Interestingly, recent studies found a causal linkage between a specific pattern of coherent gustatory cortex ensemble activity (interpreted as the emergence of a palatability computation) and the expression of simple orofacial motor patterns to a passively administered tasteant [55, 56], revealing a mechanism in gustatory cortex for top-down sensory control over simple pattern generators. In theory, the putative μ -OR modulation of local circuit dynamics in a way that amplifies palatability computations could enable simple consummatory action patterns to dominate the response repertoire, “short-circuiting” the contributions of competing pathways, and prolonging contact with food, as seen here.

Holistic appetitive-behavior sequences necessitate the integration of dissociable and sometimes competing functions—the

active, “seeking-like” repertoires that bring the organism in contact with goal-predictive cues, and, ultimately, with the goal itself; vs. the comparatively simpler consummatory patterns that accompany sensory and reward computations during commerce with the goal. The present results suggest a scheme of frontal-cortical organization in which food approach and “seeking-like” responses are preferentially represented in ventromedial prefrontal and medial orbitofrontal sites, sustained consummatory activity in insular sites, and a dissociable inhibitory-control function in ILC. In a general sense, this segregation of behavioral functions is not incompatible with recent evidence demonstrating μ -OR-driven feeding without taste-reactivity enhancement (“wanting” without “liking”) in medial prefrontal cortex and the converse pattern in posterior insular cortex [10]. It is of great interest to determine how cortical loci studied here interact as a network to merge their respective functions; this could have implications both for understanding how the network generates coherent behavior sequences, as well as how the network learns feeding-related contingencies. For example, prior work has shown that ILC participates in sugar-conditioned flavor preference learning, possibly implicating integration between insular and medial prefrontal networks [57–59]. Other important goals include determining the endogenous opioid peptides that act in this network, including those that recruit different intracellular signaling pathways [60–63]; characterizing the interaction/integration of cortical and subcortical μ -OR actions (for example, between specific cortical sites and the nucleus accumbens); and exploring potential sex differences in the behavioral functions of cortical μ -ORs. Regarding the clinical relevance of the present findings, mounting evidence from human neuroimaging studies indicates that frontal-cortical μ -ORs are involved in EDs and drug addiction [15, 16], and may contribute to an impulsivity endophenotype common to both EDs and addiction [20, 64]. Relatedly, recent studies in rats have demonstrated a strong association between high levels of action impulsivity and binge-like eating patterns [65, 66], and in agreement with the present results, have suggested that projections from the vmPFC to the nucleus accumbens shell regulate both behaviors [66]. The functional heterogeneity observed here may help provide an anatomical framework to further understand these neuroimaging and preclinical results, and to integrate them into a clinical model. For example, using food bingeing as an exemplar, supernormal μ -OR signaling events could selectively (and perhaps sequentially) drive approach toward food (VMO, ILC), degrade the ability to exhibit food restraint once there (ILC), and amplify non-homeostatic feeding once it has begun (AI/DI). Although speculative, this model provides testable predictions regarding the clinical manifestations of localized cortical μ -OR abnormalities.

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AUTHOR CONTRIBUTIONS

JLG wrote the “Methods” and “Results” with editorial assistance from BAB, assisted in writing the “Introduction” and “Discussion”, assisted in designing the experiments, carried out all experiments with assistance from EKG, RAS, and KS, and conducted all statistical analyses. EKG, RAS, and KS assisted in carrying out all experiments. BAB (PI) conceptualized the study, designed the experiments, wrote the “Abstract”, “Introduction”, and “Discussion” assisted by JLG, and supervised all other authors in their respective tasks.

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