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Central amygdala opioid transmission is necessary for increased high-fat intake following 24-h food deprivation, but not following intra-accumbens opioid administration

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Abstract

Previous research has demonstrated a dissociation of certain neural mediators that contribute to the increased consumption of a high-fat diet that follows intra-accumbens (Acb) administration of μ -opioid receptor agonists vs. 24-h food deprivation. These two models, both which induce rapid consumption of the diet, have been shown to involve a distributed corticolimbic circuitry, including the amygdala. Specifically, the central amygdala (CeA) has been shown to be involved in high-fat feeding within both opioid and food-deprivation driven models. The present experiments were conducted to examine the more specific role of CeA opioid transmission in mediating high-fat feeding driven by either intra-Acb administration of the μ -opioid agonist D-Ala2-NMe-Phe4-Glyol5-enkephalin (DAMGO) or 24-h home cage food deprivation. Injection of DAMGO into the Acb (0.25 μ g/0.5 μ l/side) increased consumption of the high-fat diet, but this feeding was unaffected by administration of opioid antagonist, naltrexone (5 μ g/0.25 μ l/side) administered into the CeA. In contrast, intra-CeA naltrexone administration attenuated high-fat intake driven by 24-h food deprivation, demonstrating a specific role for CeA opioid transmission in high-fat consumption. Intra-CeA naltrexone administration alone had no effect on baseline feeding levels within either feeding model. These findings suggest that CeA opioid transmission mediates consumption of a palatable high-fat diet driven by short-term negative-energy balance (24-h food deprivation), but not intra-Acb opioid receptor activation.

Keywords

DAMGO; Feeding; High-fat diet; Central amygdala; Nucleus accumbens; Food deprivation

1. Introduction

Feeding behavior is facilitated by a variety of factors, including homeostatic mechanisms [1,2], learned associations [3,4], and the palatable nature of the food being consumed [5]. The integration of all these factors to generate feeding behavior requires the output of a complex neural circuitry, including mesocorticolimbic areas such as the striatum,

hypothalamus, amygdala and prefrontal cortex (see [6] for review). Thorough investigation of these regions and their neurochemical mediators across different feeding models has produced the proposition that there are dissociable feeding pathways; including a homeostatic signaling pathway responding to energy balance that guides behavior toward seeking and consuming sustenance, while another pathway mediates information about palatability to guide intake of energy-rich foods beyond homeostatic needs [1,6,7]. Further dissociation of these two types of feeding behavior and the related neural circuitry is critical to understanding what role each plays in the maladaptive behaviors related to obesity.

The nucleus accumbens (Acb) is well known for its role in reward processing and translating “motivation to action” [8]. It also contains a critical site where the administration of μ -opioid agonists enhance the palatability of sucrose as measured by taste reactivity [9]. Intra-accumbens administration of the μ -opioid receptor agonist D-Ala2-NMe-Phe4-Glyol5-enkephalin (DAMGO) results in increased food consumption, an effect that is preferential in magnitude for specific diets such as sugar or those rich in fat [10,11]. Additionally, these particular experimental manipulations have implicated a role for the central amygdala (CeA) in feeding behaviors. Indeed, temporary inactivation of the CeA, but not the basolateral amygdala (BLA), blocks the increase in feeding observed following intra-Acb shell administration of the GABAA agonist muscimol, a pharmacological model that is analogous to the motivational state induced by energy deficit (moderate or severe food deprivation) [12]. In this experiment, intra-CeA inactivation produced by muscimol administration dose-dependently blocks increased chow intake following both intra-Acb muscimol administration and 24-h home cage food deprivation [12]. In addition, CeA muscimol inactivation blocks both baseline and intra-Acb DAMGO induced consumption of a high-fat diet [13,14]. However, previous experiments that investigated the involvement of CeA activity on palatable food consumption did not explore its role in mediating negative energy balance. Furthermore, considering these studies induced general inactivation (i.e., muscimol administration); it is unknown which specific neurochemical mediators are contributing to these different models of feeding.

Endogenous opioid transmission within the CeA has also been implicated in feeding behavior and is a likely candidate for mediating feeding behaviors [12,15]. Indeed, a bi-directional μ -opioid–opioid connection between the CeA and the Acb has been demonstrated [16]. For example, DAMGO administration into CeA increases feeding, but prior naltrexone administration into the Acb blocks this increase in food consumption and vice versa [16]. However, these experiments did not explore the role of CeA opioid transmission in baseline consumption of a palatable high-fat diet or following intra-Acb DAMGO administration. Glass and colleagues have shown that intra-CeA naltrexone administration reduces consumption of a “preferred” diet following 24-h food deprivation [17]. The authors suggest that the CeA may be an interface between forebrain affective systems and the hypothalamic feeding circuitry where the CeA may mediate hypothalamic activity by pairing sensory processes to the animal’s current metabolic state [17]. It is possible that this integration of sensory information about the food with the homeostatic signaling could produce an increase in the incentive value of the food, and subsequently food consumption.

The present experiments aim to extend these findings and examine how the CeA may mediate opioid-driven and energy-deficit driven feeding behaviors associated with a high-fat diet. The involvement of CeA opioid transmission was examined via intra-CeA administration of naltrexone. One group of animals was given ad libitum access to a high-fat diet following bilateral administration of naltrexone or vehicle into the CeA following 24-h home cage chow deprivation or no deprivation. A second group of animals was given ad libitum access to a high-fat diet following bilateral administration of naltrexone into the CeA immediately prior to administration of DAMGO or saline into the Acb. Feeding behavior was monitored with automated feeding chambers assessing general locomotor activity, frequency and duration of food hopper entries, and food consumption.

2. Materials and methods

2.1. Subjects

Eighteen adult male Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN) weighing 300–400 g, were housed in groups of two in Plexiglas cages in a climate-controlled colony room at a temperature of 22°C. The rats were maintained on a 12-h light–dark cycle, and all experiments were conducted during the light phase (0700–1900) between the hours of 1100 and 1300. Unless otherwise noted, rats had free access to laboratory chow and drinking water before and throughout the experiment. Experiment 1 had a total of 8 animals, while Experiment 2 had a total of 10 animals. All experimental procedures were in accord with protocols approved by the University of Missouri Institutional Animal Care and Use Committee.

2.2. Surgery

Rats were anesthetized with a mixture of ketamine and xylazine (90 mg/kg and 9 mg/kg, respectively; Sigma, St. Louis, MO). Stainless steel guide cannulas (23 ga, 10 mm) were stereotactically targeted bilaterally above the CeA (Experiment 1). For Experiment 2, each rat was implanted with 2 sets of bilateral cannulae targeted above the Acb and the CeA (Experiment 2). Therefore, each rat was implanted with two cannulae in Experiment 1 and four cannulae in Experiment 2. Coordinates for the targeted injection sites (2.5 mm below the bottom of the 10 mm guide cannula) were as follows: Acb: AP,+1.4; ML, $\pm$ 2.0; DV, -7.8 and CeA: AP, -2.0 ; ML, $\pm$ 4.0; DV: -8.3 . The coordinates for both regions were chosen to allow comparisons to earlier studies [12–14]. Guide cannulas were secured to the skull with stainless steel screws and light curable resin (Dental Supply of New England, Boston) using standard flat-skull techniques. After surgery, wire stylets were placed in the guide cannulas to prevent occlusion.

2.3. Apparatus

Behavioral assessment of feeding took place in a room separate from the colony room in eight Plexiglas (30.5 cm $\times$ 24.1 cm $\times$ 21.0 cm) automated feeding chambers (Med Associates, St. Albans, VT) running Med-PC software (Med Associates Version IV, St. Albans, VT). Rats had access to water ad libitum and approximately 35 g of palatable high-fat diet. Feeding chambers were equipped with four infrared locomotor activity beams located 6 cm apart across the length of the chamber and 4.3 cm above the floor. An

automated food hopper continuously monitored the weight (consumption) of high-fat diet. An additional infrared beam spanning the entrance of the food hopper determined the number and duration of each head entry into the hopper area. The feeding hopper and water bottle were located on the same side (opposite corners) of one chamber wall, and a removable waste tray was located beneath the bar floor. The measurements included locomotor activity (number of horizontal beam breaks), duration of hopper entry (duration of beam break at the entrance of the hopper), hopper entries (number of beam breaks at the entrance to the hopper), and amount consumed (grams).

2.4. Drug microinjection

D-Ala2, NMe-Phe4, Glyol5-enkephalin (DAMGO; Research Biochemicals, Natick, MA) and naltrexone (Sigma, St. Louis, MO) were both dissolved in sterile 0.9% saline. The vehicle control was always sterile 0.9% saline. A dose of 5 µg was chosen for naltrexone based on previous studies that demonstrated this dose to be effective in ethanol self-administration [18] and both chow and palatable diet consumption [16,17,19,20]. Rats were gently handheld and infusions were delivered with a microdrive pump (Harvard Apparatus, South Natick, MA), through polyethylene tubing (PE-10). Thirty-three-gauge 12.5-mm injectors were used, extending 2.5 mm beyond the end of the 10 mm guide cannulas. The rate of injection was 0.32 µl/min for the Acb and 0.16 µl/min for the CeA, with the total duration of infusion being 93 s, resulting in 0.5-µl and 0.25-µl volumes, respectively. One additional minute was allowed for diffusion. The current volume (0.25 µl) used for CeA infusions was much smaller than used (0.5 µl or 1.0 µl) in previous studies [16,17], which is important for preventing diffusion into the BLA and other nearby regions.

2.5. Behavioral assessment of feeding

All behavioral testing began 1-week post-surgery and occurred in the Med Associates chambers described above. Rats were placed in these chambers for 2 h daily between the hours of 1100–1300 until stable food consumption across 3 days was obtained, usually occurring within 6 days. Animals were given 2 days of sham injections over the last 2 days of the baseline period to ensure acclimation to the treatment procedure. On the first day of this acclimation procedure, a 10-mm injector was inserted and left in place for 2 min, with no volume injected. The following day, a 12.5-mm injector was inserted, and saline was administered for 93 s. In Experiment 1, naltrexone (5 µg/0.25 µl/side bilaterally) or saline was administered into the CeA after either a 24-h period of home cage chow deprivation or ad libitum access (no deprivation control). In Experiment 2, naltrexone (5 µg/0.25 µl/side bilaterally) or saline was infused into CeA, followed immediately by DAMGO (0.25 µg/0.5 µl/side bilaterally) or saline into the Acb, thus resulting in four possible treatment combinations. The test session began immediately after the last injection. Treatments were scheduled in a counter-balanced order and there were at least 2 days between each treatment session.

2.6. Specialized diet

The specialized sweetened high-fat diet was obtained from Teklad, Inc., Madison, WI. The diet contained 278.3 g/kg vitamin free casein, 4.2 g/kg DL-methionine, 100.0 g/kg sucrose, 441.2 g/kg shortening, 77.7 g/kg safflower oil, 26.3 g/kg cellulose, 53.3 g/kg mineral mix,

15.2 g/kg vitamin mix and 3.8 g/kg choline chloride. All components are expressed as weight (g). Based on energy, the diet is 6.2 kcal/g.

2.7. Histology

After behavioral testing was completed, subjects were overdosed with ketamine and xylazine and perfused transcardially with heparinized saline (200 ml), followed immediately by 500 ml of a 10% buffered formalin solution. The brains were then removed and placed in 10% formalin–20% sucrose for 1 week. Frozen serial sections (40 μ m) were collected through the entire extent of the injection site, mounted on gelatinized slides, and counter-stained with cresyl violet nissl stain. Cannulae placements were then analyzed for accuracy and data from rats with misplaced cannula were not included in the analyses. Figs. 1 and 2 show schematic representations of the injection sites with in the Acb and CeA, respectively.

3. Results

3.1. Experiment 1—The influence of intra-CeA naltrexone on the increased high-fat feeding behavior following 24-h food deprivation

All analyses were conducted using a 2-way ANOVA examining the independent variables “deprivation state” and “CeA treatment”. The ANOVA conducted on the food consumption data for Experiment 1 revealed a significant main effect of deprivation treatment ($F(1,7) = 15.19, p < 0.01$). There was a deprivation treatment $\times$ CeA treatment interaction ($F(1,7) = 19.68, p < 0.005$). In Fig. 3, post-hoc comparisons revealed that naltrexone administration into the CeA had no effect on baseline consumption by itself ($p > 0.05$), but did significantly reduce consumption following 24 food deprivation treatment ($p < 0.01$). As shown in Table 1, an ANOVA conducted on the total duration of all hopper entries across the 2 h feeding session revealed a main effect of deprivation state ($F(1,7) = 10.15, p < 0.05$), but no effect of intra-CeA naltrexone treatment ($F(1,7) = 4.21, ns$), or a deprivation state $\times$ CeA treatment interaction ($F(1,7) = 0.63, ns$). Additionally, there was no main effect of deprivation state ($F(1,7) = 0.81, ns$), intra-CeA naltrexone treatment ($F(1,7) = 1.44, ns$), nor any deprivation state $\times$ CeA treatment interaction effect ($F(1,7) = 0.98, ns$) on the number of food hopper entries. Finally, an ANOVA conducted on the activity levels revealed no effect of deprivation state ($F(1,7) = 0.10, ns$), intra-CeA naltrexone treatment ($F(1,7) = 0.23, ns$), or interaction of deprivation state $\times$ CeA treatment ($F(1,7) = 0.14, ns$).

3.2. Experiment 2—The influence of intra-CeA naltrexone on the increased high-fat feeding behavior following intra-Acb DAMGO administration

All analyses were conducted using a 2-way ANOVA examining the independent variables “intra-Acb DAMGO treatment” and “CeA treatment”. An ANOVA conducted on the food consumption data for Experiment 2 revealed a significant main effect of intra-Acb DAMGO treatment ($F(1,9) = 114.33, p < 0.001$), intra-CeA naltrexone treatment ($F(1,9) = 18.67, p = 0.001$), but no intra-Acb DAMGO treatment $\times$ CeA treatment interaction was observed ($F(1,9) = 1.10, p = 0.089$). In Fig. 4, post-hoc comparisons revealed that naltrexone administration into the CeA had no effect on baseline consumption by itself ($p > 0.05$), or following DAMGO administration ($p > 0.05$). As shown in Table 2, an ANOVA conducted on the total duration of all hopper entries across the 2 h feeding session revealed a

significant main effect of intra-accumbens DAMGO treatment ($F(1,9) = 24.60, p = 0.001$), but there was no effect of intra-CeA naltrexone treatment ($F(1,9) = 1.50, ns$) or intra-Acb DAMGO treatment $\times$ CeA treatment interaction ($F(1,9) = 1.17, ns$). An ANOVA conducted on the number of hopper entries across the entire feeding session revealed a significant main effect of intra-accumbens DAMGO treatment ($F(1,9) = 21.47, p = 0.001$), but there was no effect of intra-CeA naltrexone treatment ($F(1,9) = .96, ns$) or intra-Acb DAMGO treatment $\times$ CeA treatment interaction ($F(1,9) = 0.75, ns$). An ANOVA conducted on locomotor activity revealed significant main effect of intra-Acb DAMGO treatment ($F(1,9) = 12.38, p < 0.01$), but there was no effect of intra-CeA naltrexone treatment ($F(1,9) = 0.04, ns$) or intra-Acb DAMGO treatment $\times$ CeA treatment interaction ($F(1,9) = 0.27, ns$).

4. Discussion

The present experiments examined whether CeA opioid transmission is necessary to observe the feeding behaviors following 24-h food deprivation or intra-Acb DAMGO administration. These experiments aimed to extend previous findings and examine the specific neurochemical nature of the amygdala subregions that selectively contribute to the opioid-driven and energy-deficit driven feeding behaviors. Endogenous opioids are a likely candidate for both models of feeding considering that BLA opioids have been shown to influence reward-related behaviors associated with both drug and natural rewards, while CeA opioids have been shown to contribute to signaling pathways that mediate feeding behaviors that lead to satiety. The comparison of these two models of feeding behavior is relevant to comprehending the impact of natural reward mechanisms that contribute to the maladaptive feeding behaviors that can lead to the development of obesity. It is especially intriguing when considering that one of the major underlying causes of the current obesity trend is overconsumption of palatable tasty food in a non-deprived state. Furthering our understanding of the feeding networks that drive consumption of highly palatable diets based on their rewarding nature, rather than energy need, is of considerable importance.

The present data suggest that CeA opioid transmission is required to observe increased high-fat feeding following 24-h food deprivation treatment. However, CeA opioid transmission is not necessary to observe the exaggerated high-fat feeding following intra-Acb DAMGO administration. Indeed, animals exhibit an exaggerated increase in high-fat food consumption following 24-h home cage food deprivation and intra-Acb DAMGO administration, but this increase was completely abolished by intra-CeA naltrexone administration only following food deprivation. Further, animals exhibited an increase in food hopper entry duration following 24-h home cage food deprivation; however, intra-CeA naltrexone administration had no effect on this increased entry duration. In regard to other feeding behaviors, there were no differences among treatment groups for the number of food hopper entries or locomotor activity. Additionally, intra-Acb DAMGO administration significantly increased all other feeding behaviors, including hopper entry duration, number of entries, and locomotor activity; however, intra-CeA naltrexone administration did not alter these exaggerated behaviors.

These data suggest that a state of negative energy balance may require opioid transmission within the CeA to mediate an increase in feeding behavior since intra-CeA naltrexone

administration prevented only the increase in high-fat consumption following 24-h food deprivation and had no effect on baseline high-fat feeding behavior. Previous studies have shown a similar role of CeA opioid transmission and support the position that the CeA may relay energy balance signals to the Acb to mediate feeding behavior. Indeed, Levine and colleagues showed that intra-CeA naltrexone administration reduced Acb neural activation, as assessed by c-Fos-immunoreactivity (IR), following food deprivation, while intra-CeA DAMGO administration in free-feeding animals increased c-Fos-IR in the Acb shell [21]. In comparison, systemically administered naltrexone was also found to induce c-Fos-IR within the CeA and Acb in sated animals with free access to home cage food [22] and prevented meal induced increases in c-Fos-IR in the Acb and CeA, while having no effect on consumption levels [23]. These data suggest a distinct role of opioid transmission contributing to baseline and food deprivation feeding within the CeA-Acb pathway.

In the current study, all animals were given access to the high-fat diet prior to receiving drug treatments for the purpose of establishing stable baseline consumption. This baseline and experience are important considering the fact that only the consumption induced by 24-h food deprivation was blocked by intra-CeA naltrexone. Since intra-CeA naltrexone administration had no effect on baseline consumption, CeA opioid transmission may specifically mediate signaling related to energy balance to facilitate the increase in consumption. Other research has suggested that CeA opioid transmission is important in “focusing” incentive salience [24,25]. Considering that both acute food deprivation and intra-Acb DAMGO administration have been shown to increase salience related to food reward, including progressive ratio breakpoint [7,26] and Pavlovian-Instrumental Transfer [27,28], it is possible that CeA opioid transmission may play a critical role in encoding increased incentive salience particular to the condition of food deprivation. This endogenous opioid transmission may then have a critical role in mediating the association between interoceptive hunger state cues and the heightened affective experience with the reward. Ahn and Philips demonstrated that the presence of food initiates dopamine efflux in the Acb following food deprivation and this efflux remains during food consumption until satiety is reached. Meanwhile, inactivation of the CeA decreased basal levels of DA efflux in the Acb before food consumption and attenuated increases in DA during anticipation and consumption of the palatable diet [29]. This may suggest that CeA activity has a role in mediating the tonic release of DA in the Acb and by reducing this DA efflux, prevents the initiation of feeding. This protocol also demonstrated that introduction of a second meal when the CeA was not inactivated resulted in a significant increase in consumption and increased Acb DA release. While these experiments were not examining CeA opioid transmission specifically, the authors suggest that CeA function is critical to developing satiety and the increase in DA activity may be a mechanism by which an animal’s current state of hunger or satiety influences the approach toward incentive stimuli or food [29,30]. In particular, 24-h food deprivation may promote activation of mesolimbic dopamine circuits to amplify appetitive and consummatory behavior and CeA opioid transmission is an intermediary for this activation. Disrupting this connectivity could then block the amplified consumption behavior, while leaving baseline consumption unaffected.

Contrary to previous studies that used standard laboratory chow as the test diet, CeA opioid transmission is not critical to observe the exaggerated consumption of a high-fat diet

following intra-Acb DAMGO administration. In the present experiments, the data suggest that CeA opioid transmission has minimal contribution to hedonically-driven feeding. Previous reports from our lab suggest that palatability-induced feeding in sated animals is dependent upon basolateral amygdala activity [14] and, in particular, functional opioid receptor activity [20]. These data showed that inactivation of the BLA with muscimol blocked the increase in consumption following intra-Acb DAMGO administration, but had no effect on baseline or 24-h food deprivation feeding [14]. Interestingly, intra-BLA naltrexone administration also reduced this increased feeding, but unlike muscimol inactivation, naltrexone did not reduce consumption to baseline levels. It may be that hedonically driven feeding under sated conditions involves activity of BLA glutamate projections to the Acb and medial prefrontal cortex (mPFC) and that opioid transmission within the BLA partially contributes to the exaggerated feeding behavior following DAMGO administration. The dissociation between the feeding-related effects of BLA and CeA inactivation lend support to the idea that neural output from the CeA is required for the normal expression of the consummatory act, regardless of the manner in which feeding is elicited, whereas the BLA may be more selectively involved in higher order processing of taste and/or the hedonic aspects of ingestion. The BLA and CeA are well positioned for sensory information processing and encoding information related to rewards or stimuli that are predictive of rewards. Their particular connections provide distinct roles in mediating reward and feeding behavior in which further examination could reveal implications in understanding maladaptive behaviors observed not only in obesity, but other “goal-directed” behaviors such as exercise and drug addiction.

5. Conclusion

In summary, the present experiments provide evidence demonstrating a specific role for CeA opioid transmission in mediating homeostatic-driven, but not palatability-driven, consumption of a palatable high-fat diet. These results are particularly novel as these data are the first to show a role of opioid receptors within the CeA in mediating feeding of a high-fat diet induced by energy-deficit. The lack of influence of CeA opioid blockade on intra-Acb opioid-driven high-fat feeding behavior suggests that this behavior may depend on other areas, such as the BLA, that mediate palatability information under sated conditions [20]. Understanding the neural mediators that contribute to these two models of feeding will be critical to revealing the nature of appetitive disorders of reward, such as those that are linked to homeostatic and hedonic driven consumption of high-fat diets.

References

1. Berthoud HR. Homeostatic and non-homeostatic pathways involved in the control of food intake and energy balance. *Obesity*. 2006; 14:197–200.
2. Saper CB, Chou TC, Elmquist JK. The need to feed: homeostatic and hedonic control of eating. *Neuron*. 2002; 36:199–211. [PubMed: 12383777]
3. Petrovich GD, Ross CA, Gallagher M, Holland PC. Learned contextual cue potentiates eating in rats. *Physiology and Behavior*. 2007; 90:362–367. [PubMed: 17078980]
4. Johnson AW. Eating beyond metabolic need: how environmental cues influence feeding behavior. *Trends in Neurosciences*. 2013; 36(2):101–109. [PubMed: 23333343]

5. Berridge KC. 'Liking' and 'wanting' food rewards: brain substrates and roles in eating disorders. *Physiology and Behavior*. 2009; 97(5):537–550. [PubMed: 19336238]
6. Kelley AE, Berridge KC. The neuroscience of natural rewards: relevance to addictive drugs. *Journal of Neuroscience*. 2002; 22:3306–3311. [PubMed: 11978804]
7. Kelley AE, Baldo BA, Pratt WE, Will MJ. Corticostriatal-hypothalamic circuitry and food motivation: integration of energy, action and reward. *Physiology and Behavior*. 2005; 86:773–795. [PubMed: 16289609]
8. Mogenson GJ, Jones DL, Yim CY. From motivation to action: functional interface between the limbic system and the motor system. *Progress in Neurobiology*. 1980; 14(2–3):69–97. [PubMed: 6999537]
9. Berridge KC. Food reward: brain substrates of wanting and liking. *Neuroscience and Biobehavioral Reviews*. 1996; 20(1):1–25. [PubMed: 8622814]
10. Zhang M, Gosnell BA, Kelley AE. Intake of high-fat food is selectively enhanced by mu opioid receptor stimulation within the nucleus accumbens. *Journal of Pharmacology and Experimental Therapeutics*. 1998; 285(2):908–914. [PubMed: 9580643]
11. Will MJ, Franzblau EB, Kelley AE. Nucleus accumbens mu-opioids regulate intake of a high-fat diet via activation of a distributed brain network. *Journal of Neuroscience*. 2003; 23:2882–2888. [PubMed: 12684475]
12. Baldo BA, Alsene KM, Negron A, Kelley AE. Hyperphagia induced by GABAA receptor-mediated inhibition of the nucleus accumbens shell: dependence on intact neural output from the central amygdaloid region. *Behavioral Neuroscience*. 2005; 119:1195–1206. [PubMed: 16300426]
13. Will MJ, Franzblau EB, Kelley AE. The amygdala is critical for opioid-mediated binge eating of fat. *Neuroreport*. 2004; 15(12):1857–1860. [PubMed: 15305124]
14. Will MJ, Pritchett CE, Parker KE, Sawani AS, Ma H, Lai AY. Behavioral characterization of amygdala involvement in mediating intra-accumbens opioid-driven feeding behavior. *Behavioral Neuroscience*. 2009; 123:781–793. [PubMed: 19634936]
15. Beckman TR, Shi Q, Levine AS, Billington CJ. Amygdalar opioids modulate hypothalamic melanocortin-induced anorexia. *Physiology & Behavior*. 2009; 96(4–5):568–573. [PubMed: 19136019]
16. Kim EM, Quinn JG, Levine AS, O'Hare E. A bi-directional mu-opioid-opioid connection between the nucleus of the accumbens shell and the central nucleus of the amygdala in the rat. *Brain Research*. 2004; 1029(1):135–139. [PubMed: 15533326]
17. Glass MJ, Billington CJ, Levine AS. Naltrexone administered to central nucleus of amygdala or PVN: neural dissociation of diet and energy. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology*. 2000; 279:86–92.
18. Foster KL, McKay PF, Seyoum R, Milbourne D, Yin W, Sarma PV, Cook JM, June HL. GABA(A) and opioid receptors of the central nucleus of the amygdala selectively regulate ethanol-maintained behaviors. *Neuropsychopharmacology*. 2004; 29(2):269–284. [PubMed: 14666116]
19. Naleid AM, Grace MK, Chimukangara M, Billington CJ, Levine AS. Paraventricular opioids alter intake of high-fat but not high-sucrose diet depending on diet preference in a binge model of feeding. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology*. 2007; 293:R99–R105.
20. Parker KE, McCall JG, Will MJ. Basolateral amygdala opioids contribute to increased high-fat intake following intra-accumbens opioid administration, but not following 24-h food deprivation. *Pharmacology Biochemistry and Behavior*. 2010; 97(2):262–266.
21. Levine AS, Olszewski PK, Mullett MA, Pomonis JD, Grace MK, Kotz CM, Billington CJ. Intra-amygdalar injection of DAMGO: effects on c-Fos levels in brain sites associated with feeding behavior. *Brain Research*. 2004; 23(1015):9–14. [PubMed: 15223361]
22. Carr D, Kutchukhidze N, Park TH. Differential effects of m and k opioid antagonists on Fos-like immunoreactivity in extended amygdala. *Brain Research*. 1999; 822:34–42. [PubMed: 10082881]
23. Park TH, Carr KD. Neuronal patterns of Fos-like immunoreactivity induced by a palatable meal and meal-paired environment in saline-and naltrexone treated rats. *Brain Research*. 1998; 805:169–180. [PubMed: 9733960]

24. Mahler SV, Berridge KC. Which cue to want?: Central amygdala opioid activation enhances and focuses incentive salience on a prepotent reward cue. *Journal of Neuroscience*. 2009; 29(20): 6500–6513. [PubMed: 19458221]
25. DiFeliceantonio AG, Berridge KC. Which cue to ‘want’? Opioid stimulation of central amygdala makes goal-trackers show stronger goal-tracking, just as sign-trackers show stronger sign-tracking. *Behavioural Brain Research*. 2012; 230(2):399–408. [PubMed: 22391118]
26. Moscarello JM, Ben-Shahar O, Ettenberg A. Effects of food deprivation on goal-directed behavior, spontaneous locomotion, and c-Fos immunoreactivity in the amygdala. *Behavioural Brain Research*. 2009; 197(1):9–15. [PubMed: 18706934]
27. Wassum KM, Ostlund SB, Maidment NT, Balleine BW. Distinct opioid circuits determine the palatability and the desirability of rewarding events. *PNAS*. 2009; 106(30):12512–12517. [PubMed: 19597155]
28. Pecina S, Berridge KC. Dopamine or opioid stimulation of nucleus accumbens similarly amplify cue-triggered ‘wanting’ for reward: entire core and medial shell mapped as substrates for PIT enhancement. *European Journal of Neuroscience*. 2013; 37(9):1529–1540. [PubMed: 23495790]
29. Ahn S, Phillips AG. Modulation by central and basolateral amygdalar nuclei of dopaminergic correlates of feeding to satiety in the rat nucleus accumbens and medial prefrontal cortex. *Journal of Neuroscience*. 2002; 22(24):10958–10965. [PubMed: 12486191]
30. Everitt BJ, Parkinson JA, Olmstead MC, Arroyo M, Robledo P, Robbins TW. Associative processes in addiction and reward: the role of amygdala-ventral striatal subsystems. *Annals of the New York Academy of Sciences*. 1999; 877:412–438. [PubMed: 10415662]

Highlights

- CeA opioid blockade does not affect intra-Acb DAMGO-induced intake of a high-fat diet.
- CeA opioid blockade attenuates high-fat intake following 24-h food deprivation.
- CeA opioids mediate energy-deficit feeding, but not palatability-driven feeding of a high-fat diet.

Fig. 1. Schematic representation of the injection site within the Acb for Experiment 2. Black circles correspond to the location of the cannula tips.

Fig. 3.

Amount of food intake after 24-h food deprivation (R) or no deprivation (NR) after either naltrexone (NTX; 5 μ g/0.25 μ l per side) or saline (SAL) administration into the CeA. The *x* axis labels refer to deprivation state and intra-CeA treatment (i.e., deprivation state-CeA treatment). Values represent group means ($\pm$ SEM). Plus sign represents NR-SAL vs. R-SAL. Asterisk represents R-SAL vs. R-NTX. All levels of significance noted in comparison are $p < 0.01$.

Fig. 4.

Amount of food intake following intra-Acb DAMGO administration (DAM; 0.25 μ g/0.5 μ l) or saline (SAL) immediately after either naltrexone (NTX; 5 μ g/0.25 μ l per side) or saline (SAL) administration into the CeA. The x axis labels refer to treatment for the two regions (i.e., Acb treatment–CeA treatment). Values represent group means ($\pm$ SEM.). +++ represents SAL-SAL vs. SAL-DAM, $p < 0.001$.

Table 1

Feeding associated behaviors following 24-h food deprivation (R) or no deprivation (NR) after either naltrexone (NTX; 5 µg/0.25 µl per side) or saline (SAL) administration into the CeA. Values represent group means ($\pm$ SEM.) for a 2-h measure of total hopper entry duration time (duration of beam break at entry of hopper), number of food hopper entries (number of beam breaks at entry of hopper), and general activity (total number of beam breaks across chamber area).

Experiment 1	NR-SAL	NR-NTX	R-SAL	R-NTX
Hopper entry duration	275.0 $\pm$ 70.7	241.8 $\pm$ 52.4	563.7 $\pm$ 137.4 *	339.9 $\pm$ 73.3
Hopper entries	63.4 $\pm$ 12.5	62.8 $\pm$ 13.8	80.9 $\pm$ 15.6	61.3 $\pm$ 11.3
Activity	1032.3 $\pm$ 176.7	1036.1 $\pm$ 179.4	849.8 $\pm$ 129.0	925.5 $\pm$ 142.6

* $p < 0.05$ represents R-SAL vs. NR-SAL(each pairing represents the order of administration).

Table 2

Feeding associated behaviors following intra-Acb DAMGO administration (DAM; 0.25 µg/0.5 µl) or saline (SAL) immediately after either naltrexone (NTX; 5 µg/0.25 µl per side) or saline (SAL) administration into the CeA. Values represent group means (+/-SEM.) for a 2-h measure of total duration of hopper entries (seconds), food hopper entries (no. of beam breaks at entry of hopper), and general activity (total no. of beam breaks across chamber area).

Experiment 2	SAL-SAL	NTX-SAL	SAL-DAMGO	NTX-DAMGO
Hopper entry Duration	172.5 ± 27.5	149.1 ± 27.9	896.4 ± 228.2**	635.5 ± 85.7**
Hopper entries	53.1 ± 7.9	48.3 ± 8.6	263.3 ± 58.4**	192.6 ± 51.3**
Activity	983.8 ± 123.6	868.2 ± 108.7	1483.9 ± 293.1*	1437.8 ± 232.9*

* $p < 0.01$ represents SAL-DAMGO (each pairing represents the order of administration).

** $p < 0.001$ represents NTX-DAMGO vs. SAL-SAL (each pairing represents the order of administration).