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Opioid system in the medial prefrontal cortex mediates binge-like eating

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Abstract

Binge eating disorder is an addiction-like disorder characterized by excessive food consumption within discrete periods of time. This study was aimed at understanding the role of the opioid system within the mPFC in the consummatory and motivational aspects of binge-like eating. For this purpose, we trained male rats to obtain either a sugary, highly palatable diet (*Palatable* rats) or a chow diet (*Chow* rats) for 1h a day. We then evaluated the effects of the opioid receptor antagonist, naltrexone, given either systemically or site-specifically into the NAcc or the mPFC on a fixed ratio 1 (FR1) and a progressive ratio schedule of reinforcement for food. Finally, we assessed the expression of the genes preOpioMelanoCortin (POMC), Pro-Dynorphin (PDyn), and Pro-Enkephalin (PEnk), coding for the opioids peptides in the NAcc and the mPFC in both groups. *Palatable* rats rapidly escalated their intake by 4 times. Naltrexone, when administered systemically and into the NAcc, reduced FR1 responding for food and motivation to eat under a progressive ratio in both *Chow* and *Palatable* rats; conversely, when administered into the mPFC, the effects were highly selective for binge eating rats. Furthermore, we found a two-fold increase in POMC and a ~50% reduction in PDyn gene expression in the mPFC of *Palatable* rats, when compared to control rats; however, no changes were observed in the NAcc. Our data suggest that neuroadaptations of the opioid system in the mPFC occur following intermittent access to highly palatable food, which may be responsible for the development of binge-like eating.

Keywords

addiction; binge eating disorder; nucleus accumbens; opioid; palatability; prefrontal cortex

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Authors contribution

AB, PC and VS were responsible for the study concept and design. AB, PC, and VS performed behavioral and molecular experiments. AB and PC performed data analysis. PC, VS and LS assisted with interpretation of findings. AB and PC drafted the manuscript. PC, VS and LS provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication.

Introduction

Binge eating disorder is characterized by excessive and uncontrollable food consumption of highly palatable foods within a short period of time (Avena, Rada and Hoebel, 2008; Corwin, 2006; Cottone *et al*, 2012). Subjects experiencing binge eating describe it as a loss of control during an over-consumption of food, which leads to an uncomfortable fullness and intense feelings of disgust and embarrassment (Stein *et al*, 2007). Binge eating disorder often occurs in comorbidity with several diseases such as obesity, diabetes, cardiovascular diseases, as well as other psychiatric disorders (Javaras *et al*, 2008; Wilfley *et al*, 2011).

Significant effort has been made in attempting to isolate the factors which contribute to the development of binge eating (Cottone *et al*, 2008b; Hagan and Moss, 1997). A widely accepted hypothesis on the etiology of binge eating is based on the qualitative alternation of food palatability. Indeed, restriction to low-palatable, “safe” foods, typically driven by perceived cultural norms for thinness or health, may induce craving for more appetitive palatable foods and promotes overeating. The systematical alternation in palatability, therefore, results in a self-perpetuating vicious circle of binge/restriction pattern consumption (Laessle *et al*, 1989; Polivy and Herman, 1985), raising the question of whether binge eating disorder can be considered an addiction-‘like’ disorder (Corwin and Grigson, 2009; Cottone, Sabino, Steardo *et al*, 2008b; Cottone, Wang, Park *et al*, 2012; Epstein and Shaham, 2010).

No effective pharmacotherapies for binge eating disorder are currently available, although different experimental targets have been proposed (Cottone, Sabino, Steardo *et al*, 2008b; Cottone, Wang, Park *et al*, 2012). The opioid system has been considered to be one of the main targets for the treatment of eating disorders since the 1970s, due to early observations that opioid antagonists such as naltrexone and naloxone were able to reduce food intake (Holtzman, 1974). Later evidence showed that the opioid system was involved in the bidirectional modulation of feeding behavior, given the ability of morphine to increase appetite in food deprived and non-deprived rats (Sanger and McCarthy, 1980). Since these initial observations, a prominent role of the opioid system in mediating food palatability has been clarified and extensive evidence has indicated that the nucleus accumbens (NAcc) represents a key region mediating these effects (Le Merrer *et al*, 2009). More recent studies have suggested that the opioid modulation of hedonic food consumption in the NAcc is part of a more complex network, which involves several brain structures including the prefronto-cortical regions (Mena, Sadeghian and Baldo, 2011).

Even though an extensive line of research emphasizes the primary role of the opioid system in the modulation of palatability and hedonic feeding, the specific brain area in which the opioid system mediates binge-like eating is still unknown.

Therefore, the aim of this study was to determine whether the opioid antagonist naltrexone, administered systemically, was able to suppress the consumption of and the motivation to obtain highly palatable food in a rat binge-like eating model. For this purpose, we used a newly developed operant model where rats self-administer a highly palatable, sugary diet under limited access conditions (1 h/day), mimicking the consummatory and motivational

features observed in binge eating disorder (Cottone, Wang, Park *et al*, 2012). We then went on to determine which brain area was responsible for naltrexone's systemic effects in suppressing the consumption of and the motivation to obtain the sugary, highly palatable diet. For this purpose, we microinfused site specifically the opioid antagonist into the NAcc shell and mPFC. Finally, we evaluated the expression of the genes preOpioMelanoCortin (POMC), Pro-Dynorphin (PDyn), and Pro-Enkephalin (PEnk), coding for the opioids peptides in the NAcc and the mPFC following a history of binge-like eating.

Material and Methods

Subjects

Male Wistar rats (*n*=70), 41–47 days old upon arrival (Charles River, Wilmington, MA) were housed in wire-topped, standard plastic cages in a 12:12 h reverse light cycle (lights off at 10:00 h), in a humidity- (60%) and temperature-controlled (22 °C) vivarium. Upon arrival, rats had access to corn-based chow (Harlan Teklad LM-485 Diet 7012 (65% (kcal) carbohydrate, 13% fat, 21% protein, 341 cal/100 g); Harlan, Indianapolis, IN) and water *ad libitum* at all times. Procedures adhered to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH publication number 85-23, revised 1996) and the *Principles of Laboratory Animal Care* (http://www.nap.edu/readingroom/bookslabrats), and were approved by Boston University Institutional Animal Care and Use Committee (IACUC). The experimental procedures did not involve food or water restriction/deprivation, unless otherwise specified.

Drugs

Naltrexone, (5α)-17-(Cyclopropylmethyl)-4,5-epoxy-3,14-dihydromorphinan-6-one hydrochloride, was purchased from Abcam (Cambridge, MA). Naltrexone was freshly dissolved in isotonic filtered saline (0.9%) on the day of the test. Naltrexone was administered subcutaneously (0, 0.03, 0.1 and 0.3 mg/kg, 1 ml/kg) 30 minutes before the session and site-specifically into the NAcc shell and mPFC (0, 5 and 25 ug/side, bilateral) immediately before the session. Doses and pretreatment times were based on the literature (Bodnar *et al*, 1995; MacDonald, Billington and Levine, 2003; Williams and Broadbridge, 2009).

Operant binge-like eating procedure

Subjects were trained to self-administer food and water in individual operant test chambers described in details in (Blasio *et al*, 2012; Cottone, Wang, Park *et al*, 2012). For further details, see Supplementary Materials and Methods.

Training—The operant model of binge-like eating was performed as previously described (Cottone, Wang, Park *et al*, 2012). Rats (*n*=70) were fed the standard Harlan Teklad diet in their home cage. After a period of acclimation, food was replaced with an AIN-76A-based diet, hereafter referred to as 'Chow A/I' (5TUM diet formulated as 4–5 g extruded pellets, 65.5% (kcal) carbohydrate, 10.4% fat, 24.1% protein, 330 cal/100 g; TestDiet, Richmond, IN). Rats were trained to acquire operant self-administration for food (45-mg precision food pellets (Chow A/I)) and water (100 μl) under a fixed ratio 1 schedule of reinforcement

(Cottone *et al*, 2009). Dispenser delivered a 45-mg precision pellet which is identical to the home cage ~5g extruded diet, in order to ensure that *Chow* rats' food intake was driven only by homeostatic needs (Cottone, Sabino, Roberto *et al*, 2009; Cottone *et al*, 2008a). Daily sessions were performed before the dark cycle onset and were 1 h in duration.

Testing—After stable baseline performances in the 1 h self-administration sessions, the testing procedure was initiated. Rats, matched for body weight, daily food intake, feed efficiency, as well as water and food responding in the self-administration, were assigned to either a "*Chow*" control group, which in the operant boxes received the same 45-mg chow pellets offered in the training phase, or a "*Palatable*" group, which instead received a nutritionally complete, chocolate-flavored, high sucrose (50% kcal) AIN-76A-based diet, comparable in macronutrient composition and energy density to the chow diet (chocolate-flavored Formula 5TUL: 66.7% [kcal] carbohydrate, 12.7% fat, 20.6% protein, metabolizable energy 344 cal/100g; formulated as 45 mg precision food pellets; TestDiet, Richmond, IN). This chocolate-flavored diet is strongly preferred by all rats (Cottone, Sabino, Steardo *et al*, 2008a, b). Sessions were conducted daily.

Progressive ratio schedule of reinforcement for food

Following stabilization of intake in the binge-like eating procedure, rats (n=53) were trained in progressive ratio for food reinforcers (45-mg chow-precision pellets for *Chow* group and 45-mg chocolate-flavored, high sucrose-precision pellet for *Palatable* group). Progressive ratio schedule of reinforcement for food was performed as described previously (Cottone, Sabino, Roberto *et al*, 2009; Cottone, Sabino, Steardo *et al*, 2008a; Sabino *et al*, 2011). For further details, see Supplementary Materials and Methods. At the end of each session, subjects were returned to their home cage where Chow A/I was always available *ad libitum*; sessions were conducted daily.

Intracranial surgeries and microinfusion procedure

Intracranial surgeries—Following stabilization of intake in the operant sessions, rats were implanted with intracranial cannulas. Stereotaxic surgeries were performed as previously described (Cottone *et al*, 2007; Iemolo *et al*, 2012; Sabino *et al*, 2007). For further details, see Supplementary Materials and Methods. The cannula coordinates used for NAcc shell and mPFC were A/P +1.06 mm, M/L ± 0.75 mm, D/V −6.0 mm and A/P +3.2 mm, M/L ±0.65 mm, D/V −3.5 mm, respectively. The interaural bar was set at flat skull (dorsal/ventral: bregma = lambda); coordinates were based on the atlas of Paxinos & Watson (Paxinos, 1986). A stainless steel dummy stylet (Plastics One, Inc., Roanoke, VA, USA) maintained patency. After surgery, rats were allowed to recover from the surgical procedure for 1 week before the experimental procedure began.

Microinfusion procedure—For the microinfusion of naltrexone, the dummy stylet was removed from the guide cannula and replaced with a stainless steel injector projecting 1.0 mm for NAcc shell and 1.5 mm for mPFC beyond the tip of the guide cannula; the injector was connected via a PE 20 tubing to a Hamilton microsyringe driven by a microinfusion pump (Kd Scientifics/Biological Instruments, Holliston, MA, USA). Microinfusions delivered a 0.5 µl volume over 2 min; injectors were left in place for an additional minute to

minimize backflow. Treatments were given in full Latin square designs, with 1–3 intervening treatment-free test days, in which the food intake returned to baseline levels. Rats were given 3 days of acclimation to daily sham injections, prior to the beginning of drug treatments. Cannula placement (Fig. 4) was verified at the conclusion of all testing by microinfusing India Ink (0.5 μl over 2 minutes). Slices of 40 μm were collected using a cryostat and placements were verified under a microscope. Of 68 rats used for the microinfusion studies, 3 were excluded for procedural reasons, which included loss or occlusion of cannulae, or inability to maintain stable performance. In 14 of the remaining 65 rats, the position of the cannula was outside the target site.

Quantitative Real-Time PCR

One cohort of *Chow* and *Palatable* rats (n=15) was used for the quantification of the POMC, PDyn, and PEnk peptides mRNA. Animals were sacrificed 24 h after the last daily binge-like eating session. Punches of the medial prefrontal cortex include both the prelimbic and the infralimbic regions. Punches of the nucleus accumbens include both shell and core regions. Procedures were performed as described previously (Sabino *et al*, 2009). For details, see Supplementary Materials and Methods.

Statistical Analysis

The effects of naltrexone on food intake, water intake, and breakpoint were analyzed using two-way mixed design ANOVAs, with Diet History as a between-subjects factor and Treatment as a within-subject factor, followed by repeated measures one-way ANOVAs. The effects of Diet History on mRNA levels were analyzed using unpaired Student's *t*-tests. Variables that failed the test for normality were analyzed as ranked (Akritas, 1990). The statistical packages used were Instat 3.0 (GraphPad, San Diego, CA) and Systat 11.0 (SPSS, Chicago, IL).

Results

Effects of systemic administration of the opioid antagonist naltrexone on operant binge-like eating

Systemic administration of naltrexone dose-dependently reduced FR1 responding for food in both *Chow* and *Palatable* groups (Figure 1A; Treatment: $F(3,54)=25.00$, $p<0.001$; Diet History X Treatment: $F(3,54)=0.64$, *n.s.*) One-way ANOVAs confirmed the effect of drug treatment in both *Chow* (Treatment: $F(3,27)=7.62$, $p<0.0008$) and *Palatable* rats (Treatment: $F(3,27)=16.78$ $p<0.0001$). Post hoc analysis revealed that, while the 0.03 mg/kg dose was ineffective, both the 0.1 and 0.3 mg/kg doses significantly reduced food self-administration in the two groups, when compared to vehicle condition. Moreover, water intake was affected by the treatment (Table 1; Treatment: $F(3,54)=8.46$, $p<0.0001$; Diet History X Treatment: $F(3,54)=0.76$, *n.s.*). One-way ANOVAs confirmed the drug treatment effect in both *Chow* (Treatment: $F(3,27)=4.97$, $p<0.007$) and *Palatable* (Treatment: $F(3,27)=3.76$, $p<0.022$) rats. Post hoc analysis revealed a significant water intake reduction following the administration of the 0.1 and 0.3 mg/kg doses in *Chow* rats and the 0.3 mg/kg dose in *Palatable* rats, when compared to vehicle condition.

Effects of systemic administration of the opioid antagonist naltrexone on a progressive ratio schedule of reinforcement for food

Naltrexone, administered systemically, dose-dependently decreased the breakpoint in both *Chow* and *Palatable* rats (Figure 1B; Treatment: $F(3,51)=41.31$, $p<0.0001$; Diet History X Treatment: $F(3,51)=1.96$, *n.s.*). Following a significant main effect of treatment (*Chow* group; Treatment: $F(3,27)=5.99$, $p<0.003$; *Palatable* group; Treatment: $F(3,24)=6.87$, $p<0.002$), post hoc analysis revealed that the 0.3 mg/kg dose significantly reduced the breakpoint in both groups.

Effects of naltrexone microinfusion into the NAcc shell on operant binge-like eating

Naltrexone microinfusion into the NAcc dose-dependently decreased responding for food in both the *Chow* and *Palatable* group (Figure 2A; Treatment: $F(2,32)=10.76$, $p<0.0001$; Diet History X Treatment: $F(2,32)=4.36$, $p<0.02$). One-way ANOVAs revealed a drug treatment effect in both *Chow* (Treatment: $F(2,18)=5.72$, $p<0.01$) and *Palatable* rats (Treatment: $F(2,18)=5.344$, $p<0.02$) respectively. Furthermore, post hoc analysis revealed significant reductions in food self-administration following treatment with the highest dose (25 μg) in both *Chow* and *Palatable* groups. Water consumption was not affected by the drug treatment (Table 1; Treatment: $F(2,32)=2.48$, *n.s.*; Diet History X Treatment: $F(2,32)=0.65$, *n.s.*).

Effects of naltrexone microinfusion into the NAcc shell on a progressive ratio schedule of reinforcement for food

When naltrexone was site-specifically microinfused into the NAcc, a significant overall reduction of the breakpoint in both the *Chow* and *Palatable* group was observed (Figure 2B; Treatment: $F(2,30)=16.72$, $p<0.0001$; Diet History X Treatment: $F(2,30)=5.22$, $p<0.01$). This result was confirmed by the one way ANOVAs performed individually on each of the two groups (*Chow* group; Treatment: $F(2,16)=6.11$, $p<0.01$; *Palatable* group; Treatment: $F(2,14)=10.62$, $p<0.001$). Post-hoc analysis revealed a significant reduction of the breakpoint in both groups when the 5 and 25 μg doses were microinfused. The reduction of the breakpoint was comparable in magnitude between *Chow* and *Palatable* rats (50.8% and 53.2%, when compared to vehicle conditions, respectively).

Effects of naltrexone microinfusion into the mPFC on operant binge-like eating

Naltrexone site-specifically microinfused into the mPFC differentially affected food responding in *Chow* and *Palatable* rats, as revealed by the significant interaction (Figure 3A; Treatment: $F(2,30)=4.77$, $p<0.02$; Diet History X Treatment: $F(2,30)=5.08$, $p<0.01$). Indeed, while naltrexone did not affect responding for chow in *Chow* rats (Treatment: $F(2,12)=0.68$, *n.s.*), it dose-dependently reduced binge-like eating in *Palatable* rats (Treatment: $F(2,18)=9.25$, $p<0.002$), with post hoc analysis showing significant reduction following the 25 μg dose, when compared to vehicle condition. Therefore, naltrexone, microinfused into the mPFC, selectively affected binge-like eating in *Palatable* rats, without affecting feeding in control rats. In addition, naltrexone had no effect on water intake (Table 1; Treatment: $F(2,30)=1.89$, *n.s.*; Diet History X Treatment: $F(2,30)=0.69$, *n.s.*).

Effects of naltrexone microinfusion into the mPFC on a progressive ratio schedule of reinforcement for food

A two-way ANOVA performed on the breakpoint of *Chow* and *Palatable* rats following microinfusion of naltrexone into the mPFC revealed a main effect of drug treatment (Figure 3B; Treatment: $F(2,30)=9.057$, $p<0.001$; Diet History X Treatment: $F(2,30)=1.84$, *n.s.*). However, although the one-way ANOVA analysis revealed an effect of drug treatment in the *Chow* group (Treatment: $F(2,18)=4.43$, $p<0.027$), following post-hoc analysis neither the 5 μg nor the 25 μg dose significantly differed from the vehicle condition. Likely, the treatment's significant effect indicated by the ANOVA was driven by a trend toward an increased breakpoint following the 5 μg dose, when compared to vehicle condition. On the other hand, in the *Palatable* group a significant main effect of drug treatment could be observed (Treatment: $F(2,12)=5.31$, $p<0.02$), and the highest dose microinfused into the mPFC significantly reduced the breakpoint, when compared to vehicle condition.

Quantitative Real-Time PCR

Quantitative real-time PCR showed that, 24 h after the last food self-administration session, no significant differences in POMC, PDyn, and PEnk expression between *Chow* and *Palatable* rats were observed in the NAcc (Figures 5A, 5B, and 5C). However, POMC mRNA levels were significantly higher in the PFC of *Palatable* rats, when compared to *Chow* rats (117.9% increase; Figure 5D). In addition, PDyn expression levels in the PFC of *Palatable* rats were significantly lower in comparison to *Chow* rats (49.3% reduction; Figure 5E). No difference between the two groups was observed in the PEnk mRNA levels in the PFC (Figure 5F).

Discussion

In this study we show that the opioid antagonist naltrexone, systemically administered, non-specifically decreased the consumption and the motivation to obtain food, as well as reduced the intake of water in both rats self-administering a regular chow diet and rats bingeing on a highly palatable diet. Importantly, while the effects on both chow and palatable food intake were maintained when naltrexone was microinfused into the NAcc shell, the opioid antagonist selectively decreased the consumption and the motivation to obtain highly palatable food, but not regular chow, when microinfused into the mPFC. Furthermore, confirming the selectivity of the behavioral effects observed following the microinfusion of naltrexone into the mPFC, the mRNA expression of POMC and PDyn was dysregulated in the mPFC, but not in the NAcc, of binge eating rats in comparison to control rats. No effect was observed in the gene expression of PEnk in either area.

Systemically administered naltrexone, therefore, dose-dependently decreased food consumption of both *Palatable* and *Chow* rats. Systemic drug treatment also reduced the motivation to work, in order to obtain both the chow and the palatable diet in a progressive ratio schedule of reinforcement, a validated behavioral paradigm utilized to assess the motivational strength to acquire reinforcers (Cottone, Sabino, Roberto *et al*, 2009; Cottone, Sabino, Steardo *et al*, 2008a). Following subcutaneous administration of the highest dose naltrexone, the magnitude of the maximal effects in the reduction of FR1 responding and the

breakpoint of a progressive ratio schedule of reinforcement was similar in the two groups (FR1: 58.2% and 54.0%; progressive ratio: 40.5% and 43.3%, when compared to vehicle conditions in *Chow* and *Palatable* rats, respectively). Therefore, the effects of systemic naltrexone on food intake likely involved a suppression of both homeostatic and hedonic feeding behavior (Le Merrer, Becker, Befort *et al*, 2009). Interestingly, the effects of subcutaneous administration of naltrexone were not selective for food, since drug treatment also decreased water intake in both control and binge eating rats. Altogether, these initial observations were suggesting a general suppressive effect on ingestive behavior, following systemic administration with the opioid antagonist (Frenk and Rogers, 1979).

In this study we wanted to determine whether opioid receptors in the NAcc shell mediated the consummatory and motivational aspects of binge-like eating. Indeed, the opioid system in this area has been proposed to be involved in the modulation of the rewarding properties of food (Carlezon, Devine and Wise, 1995). Here we show that naltrexone microinfused into the NAcc shell decreased not only binge-like eating of the highly palatable diet, but also the intake of regular chow. A similar outcome was obtained when we tested the effects of naltrexone microinfusion into the NAcc shell on the breakpoint of a progressive ratio schedule of reinforcement for food. Indeed, drug treatment indiscriminately decreased the motivation to obtain food in both binge eating and control rats. These findings suggest that opioid receptors within the NAcc shell exert a general modulation of feeding behavior and reinforcing efficacy of food, independently from the type or from the incentive salience of the diet. In support of this hypothesis, Kelley and colleagues have shown that blockade of μ-opioid receptors within the NAcc decreases the intake of both a standard chow diet and a sucrose solution (Kelley, Bless and Swanson, 1996). Contrarily to what we observed following systemic administration of naltrexone, NAcc shell microinfusion of the drug did not affect water intake, suggesting that opioid receptors in this brain region are specifically involved in the modulation of feeding behavior, instead of more generally in ingestive behavior, or that higher doses are needed to suppress water intake.

We also investigated whether the opioid system within the mPFC was important in mediating binge-like eating of highly palatable food. In our study, mPFC microinfusion of the opioid antagonist selectively and dose-dependently decreased both the consumption and the motivation to obtain the highly palatable diet in binge eating rats, without affecting the intake of regular chow in control rats. Water intake was not affected by drug treatment in either group, suggesting that the effects are selective for feeding behavior. Fronto-cortical areas of the brain have been implicated in the modulation of feeding behavior (Moran and Westerterp-Plantenga, 2012). A recent report has also shown that μ-opioid receptors within the mPFC play an important role in driving overeating (Mena, Sadeghian and Baldo, 2011).

It is important to discuss the alternative interpretation that the effects of naltrexone may result from its rapid diffusion throughout the brain and periphery, contrarily to other quaternary derivatives opiate antagonists (Vaccarino, Bloom and Koob, 1985; Vaccarino *et al*, 1985) which have a low diffusion rate (Schroeder *et al*, 1991). Contrary to this interpretation there is the evidence that, in this study, naltrexone microinfused into two different brain areas (NAcc and PFC) in *Chow* rats exerted differential effects. Moreover, time course analysis of food responding revealed that naltrexone injected into the mPFC

decreased food responding in the *Palatable* rats after only 6 minutes following microinfusion (*M*±SEM: 84.3±7.5 vs. 75.3±6.6, veh vs. 25 μg/side, respectively, $p<0.05$). Because of the short period of time, the alternative interpretation that the observed effect could result either from a CNS-wide or peripheral blockade of opioid receptors is highly unlikely. Furthermore, in support of the validity of the data obtained in this study, literature extensively reports the effects of naltrexone microinfused in specific areas of the brain (Bodnar *et al*, 2005). Nonetheless, the hypothesis that the effect of naltrexone may be also dependent on a slight diffusion in brain areas contiguous to Nacc shell or PFC, cannot be excluded.

An alternative interpretation of the lack of effects on food intake following naltrexone microinfusion into the prefrontal cortex of *Chow* rats is that the observed effect could result from a concomitant blockade of mu and kappa opioid receptors. Although the two systems have been demonstrated to exert opposite modulatory effects in multiple processes including reward, they have been shown to modulate homeostatic feeding (like the food intake in *Chow* animals of this study) in a similar manner. Both mu and kappa opioid receptor activation has been demonstrated to increase food intake, whereas their blockade has been shown to exert anorectic effects (Cooper, Jackson and Kirkham, 1985; Gosnell, Levine and Morley, 1986) Therefore, our findings suggest a differential role in the modulation of eating behavior exerted by the opioid system in the NAcc and the mPFC; while NAcc opioid receptors seem to be involved in a general modulation of feeding, independently from the type of food ingested (Kelley, Bless and Swanson, 1996), the mPFC opioid system only seems to be recruited following a history of limited access to a sugary, palatable diet, when rats lose inhibitory control over food. This hypothesis is in accordance with the higher cognitive function and complex control of reward evaluation exerted by the mPFC.

The hypothesis of a more generalized, not food specific role of NAcc opioid receptors in the mediation of feeding behavior, and the selective recruitment of the mPFC was supported by gene expression analysis of POMC, PDyn, and PEnk. No significant difference in the expression of the three genes was observed in the NAcc, when comparing the binge eating and control rats. Conversely, in the mPFC, binge eating rats showed a more than two-fold increase in the POMC mRNA levels, accompanied by a ~50% reduction in the mRNA levels of PDyn, when compared to control chow rats.

POMC and PDyn genes have been shown to be expressed in both of these brain areas (Leriche, Cote-Velez and Mendez, 2007; Taqi *et al*, 2011; Ziolkowska *et al*, 2006). (However, evidence demonstrates that opioid peptides released in mPFC can also originate from cell bodies projecting from different brain regions (i.e. ventral tegmental area (Garzon and Pickel, 2004)), which raises the possibility that the effects observed in this study following naltrexone microinfusion within the mPFC may be unrelated to the variation in POMC and Pdyn expression in that same brain region. POMC is the precursor of endorphins which preferentially bind μ (but also δ) opioid receptors (Mansour *et al*, 1995), while PDyn is the precursor of dynorphins which preferentially bind κ-opioid receptors (Day *et al*, 1998). Extensive evidence has suggested an opposing role of μ- and κ-opioid receptors in the modulation of a variety of processes in the brain including analgesia, tolerance, memory processes, and reward (Pan, 1998; Woolley *et al*, 2007). In particular and consistent with

this hypothesis, μ-opioid receptors have been extensively proven to mediate the rewarding properties of food and some drugs of abuse (Zhang and Kelley, 2000); on the other hand, κ-opioid receptors have been demonstrated to mediate their aversive and dysphoric effects, and have been proposed as a part of an "anti-reward" system (Koob and Le Moal, 2001). More importantly, in the context of this study, the pharmacological activation of either μ- or κ-opioid receptors within the prefrontal cortex has been demonstrated to exert opposing rewarding effects: microinfusions of a selective μ-opioid receptor agonist induce place preference, whereas microinfusions of a κ-opioid receptor agonist produce place aversion (Bals-Kubik *et al*, 1993).

An important point of discussion is whether changes in the mRNA expression observed in this study are dependent on differences in cumulative caloric intake or body weight between *Chow* and *Palatable* rats. Although food intake and body weight were not recorded in the cohort of animals used for the quantitative Real-Time PCR, we have previously demonstrated that the binge-like eating procedure used here does not influence cumulative food intake nor body weight. Indeed, binge eating rats show excessive intake during the 1h access to the highly palatable diet, but compensate during the remaining 23 hours of the day by undereating the regular chow diet (Cottone, Wang, Park *et al*, 2012). This aberrant pattern of intake therefore does not result in differing cumulative caloric intake or body weight between binge eating and control rats (Cottone, Wang, Park *et al*, 2012).

Although similarities between the overeating/restriction pattern in binge eating disorder and the intoxication/withdrawal pattern of drug abuse have been proposed (Epstein and Shaham, 2010), whether the animal model used in this study could also be useful for the of study the negative symptomatology associated with withdrawal from the highly palatable food is unknown. The increased expression of POMC ("pro-reward" system) and the decreased expression of Pdyn ("anti-reward" system) observed here following 24h withdrawal from the palatable diet suggest that the animals likely do not experience a negative emotional state. However, to address this important aspect of diet cycling, further studies assessing emotionality and the potential involvement of stress systems (e.g. corticotropin-releasing factor) will be needed. Therefore, the differential alterations in the gene expression of POMC and Pdyn observed in mPFC may indeed be interpreted as a general potentiation of the rewarding properties of palatable food, which may be the consequence of or drive binge eating in these subjects.

Altogether, the results of this study support the hypothesis that the opioid system in prefronto-cortical regions of the brain is involved in the control of feeding behavior and expand it to the specific context of the maladaptive excessive intake of highly palatable food observed in binge eaters (Mena, Sadeghian and Baldo, 2011). Fronto-cortical regions of the brain play a major role in reward evaluation and decision making (Clark *et al*, 2008); extensive literature demonstrates that subjects afflicted by addiction and binge eating disorder show dysfunctions in the mPFC, which are associated with altered reward evaluation (Boeka and Lokken, 2011). Our behavioral, pharmacological, and molecular observations, therefore, support the hypothesis that the opioid system is a key mediator of hedonic feeding (Nathan and Bullmore, 2009) and suggest that neuroadaptations of the

opioid system in the mPFC may be responsible for the hyper-evaluation of highly palatable foods, leading to the loss of control over eating.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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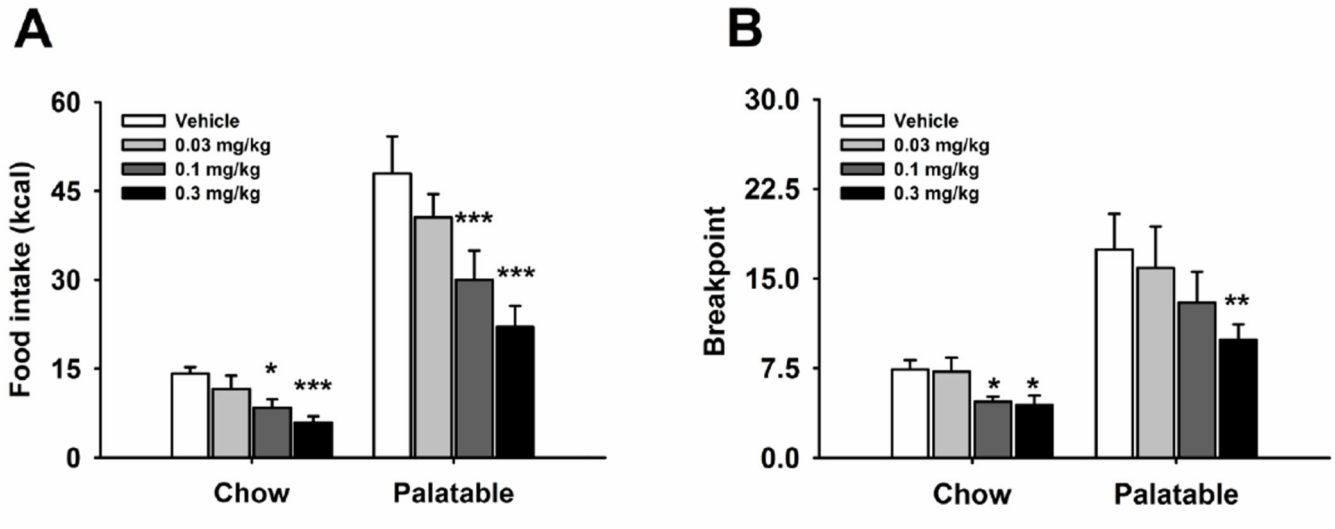

Figure 1.

Effects of systemic treatment with naltrexone (0, 0.03, 0.1, 0.3 mg/kg, subcutaneously) on **(A)** food self-administration (n=20) and **(B)** breakpoint on a progressive ratio schedule of reinforcement (n=19) in male Wistar rats. Panels represent M±SEM. Symbols denote: * significant difference from vehicle condition $p<0.05$, ** $p<0.01$, *** $p<0.001$ (Student Newman–Keuls test).

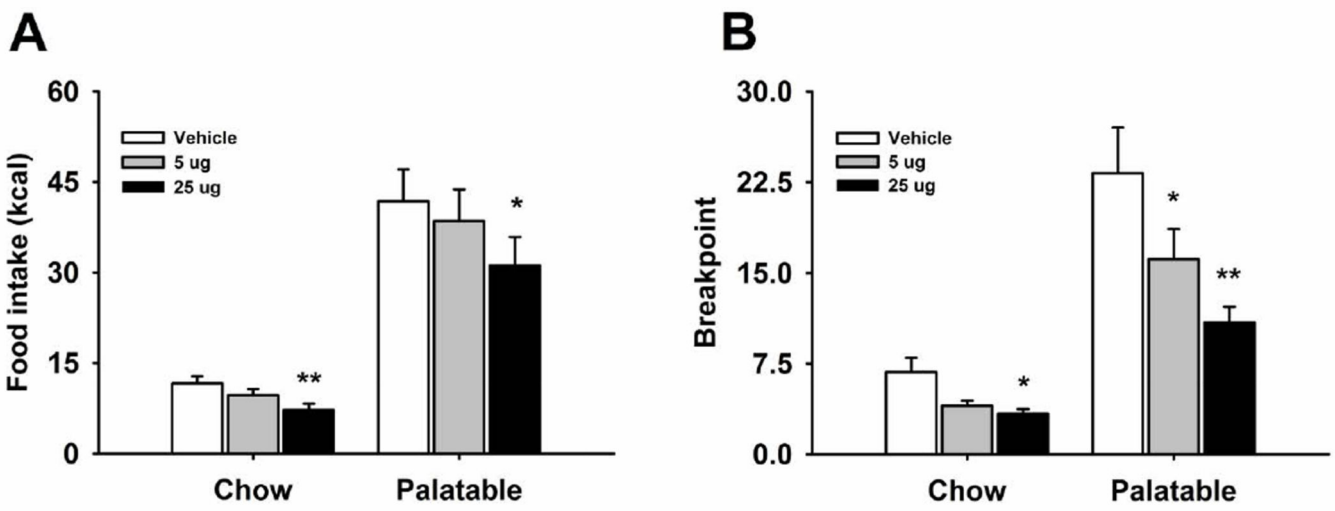

Figure 2.

Effect of microinfusion with naltrexone (0, 5, 25 μg/side) in NAcc shell on **(A)** food self-administration (n=18) and **(B)** breakpoint on a progressive ratio schedule of reinforcement (n=17) in male Wistar rats. Panel represents M±SEM. Symbols denote: * significant difference from vehicle condition $p<0.05$, ** $p<0.01$ (Student Newman–Keuls test).

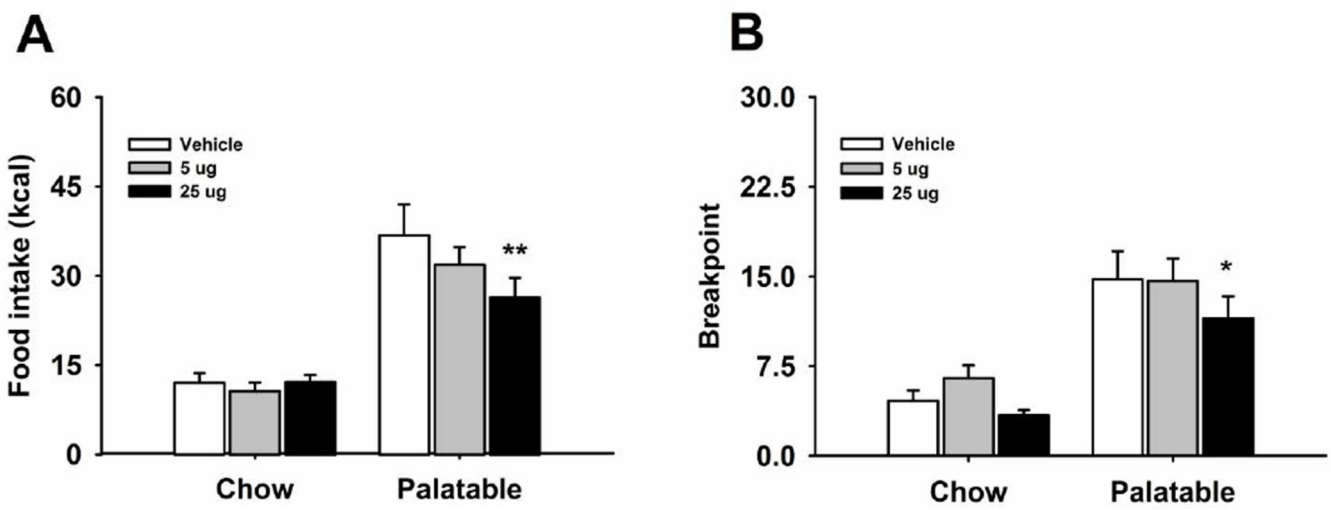

Figure 3.

Effect of microinfusion with naltrexone (0, 5, 25 μg/side) in mPFC on (**A**) food self-administration (n=17) and (**B**) breakpoint on a progressive ratio schedule of reinforcement (n=17) in male Wistar rats. Panel represents M±SEM. Symbols denote: * significant difference from vehicle condition $p<0.05$, ** $p<0.01$ (Student Newman–Keuls test).

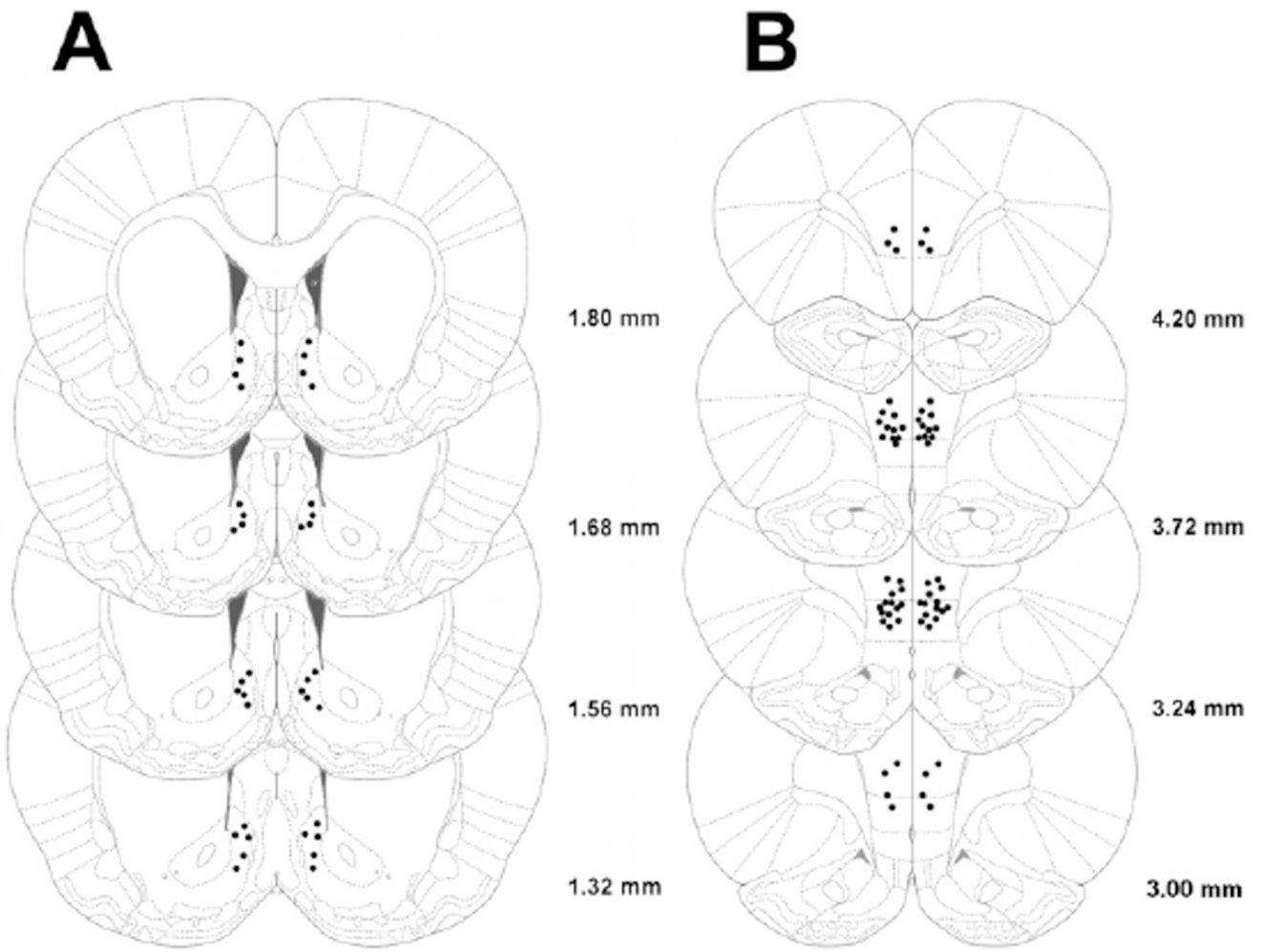

Figure 4.

Drawing of coronal rats' brain slices. Dots represent the injection sites in **(A)** NAcc shell and **(B)** mPFC included in the data analysis.

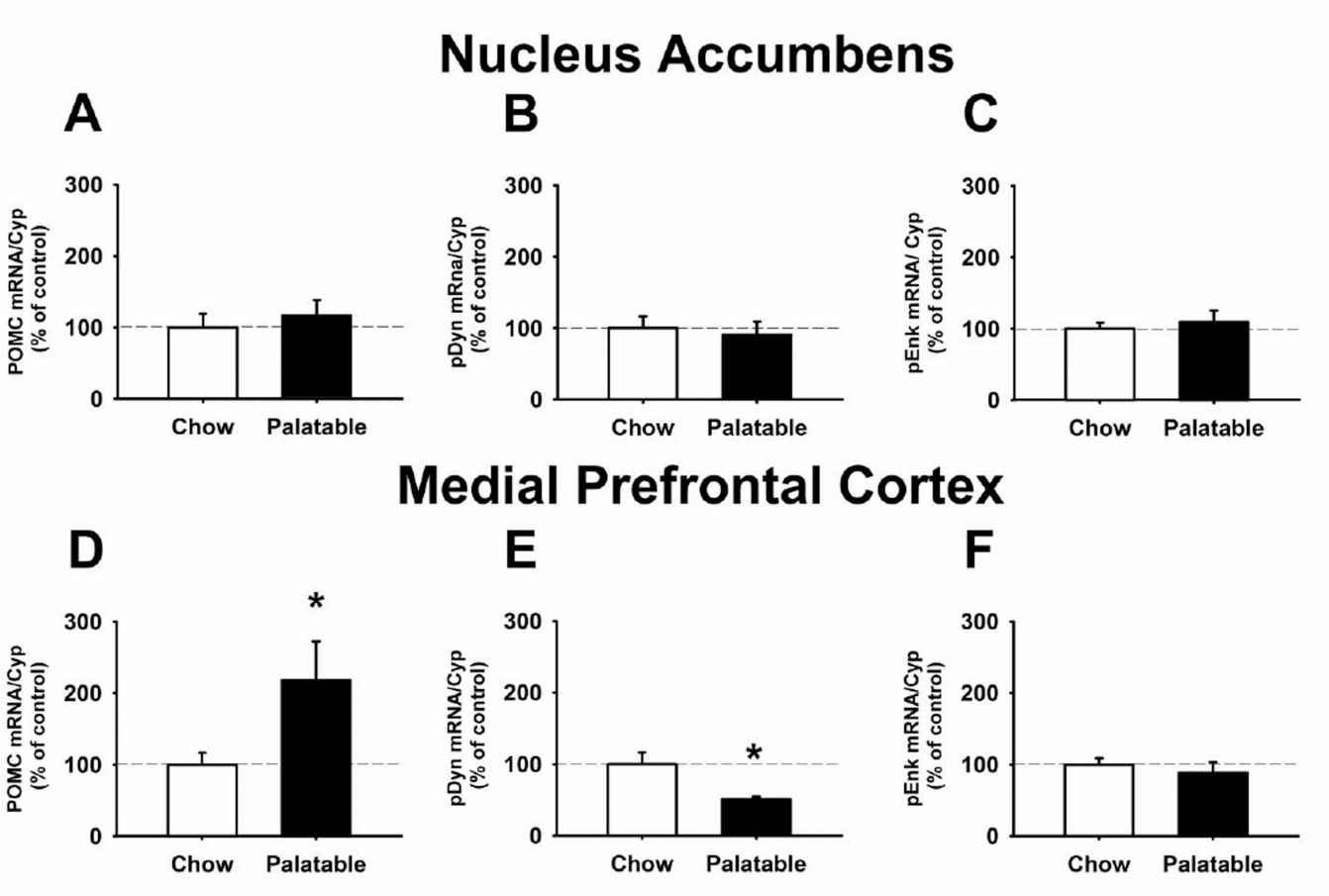

Figure 5.

POMC, PDyn, and PEnk mRNA expression in the NAcc (**A, B** and **C**) and in the mPFC (**D, E** and **F**) of male Wistar rats. Brain areas were collected 24 h after the last daily binge-like eating session. Data represent *M*±SEM expressed as percent of *Chow* group; * $p<0.05$ vs *Chow* group (Student's *t*-test).

Table 1

Effects of naltrexone administration on water intake

Effects of naltrexone on water intake following subcutaneous (*n*=20), NAcc shell (*n*=18) or mPFC (*n*=17) administration in male Wistar rats. Values show log-normal values of water intake espressed as *M*±SEM. Bracketed values show the backtransformed *M*±SEM.

Water intake, ln(ml) [ml]			
Treatment		**Chow**	**Palatable**
Systemic			
	Vehicle	1.95-0.06 [7.0±0.5]	1.70-0.14 [5.4±0.8]
	0.03 mg/kg	1.60-0.17 [5.0±0.9]	1.45-0.17 [4.3±0.8]
	0.1 mg/kg	0.99-0.41 [2.7±1.4] *	1.24-0.21 [3.5±0.8]
	0.3 mg/kg	0.82-0.22 [2.3±0.6] *	0.99-0.32 [2.7±1.0] *
NAcc Shell			
	Vehicle	1.99-0.10 [7.3±6.1]	2.08-0.19 [8.0±1.7]
	5 ug	1.80-0.13 [6.1±0.9]	1.51-0.42 [4.5±2.3]
	25 ug	1.90-0.15 [6.7±1.1]	1.79-0.16 [6.0±1.1]
mPFC			
	Vehicle	1.87-0.18 [6.5±1.3]	1.89-0.15 [6.6±1.1]
	5 ug	1.87-0.14 [6.5±0.9]	1.63-0.16 [5.1±0.9]
	25 ug	1.55-0.13 [4.7±0.6]	1.64-0.19 [5.2±1.1]

Symbols denote: * significant difference from vehicle condition $p<0.05$ (Student Newman–Keuls test).