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CRITICAL ROLE OF NMDA BUT NOT OPIOID RECEPTORS IN THE ACQUISITION OF FAT-CONDITIONED FLAVOR PREFERENCES IN RATS

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Abstract

Animals learn to prefer flavors associated with the intake of dietary fats such as corn oil (CO) solutions. We previously reported that fat-conditioned flavor preferences in rats were relatively unaffected by systemic treatment with dopamine D1 and D2 antagonists. The present study examined whether systemic opioid (naltrexone, NTX) or NMDA (MK-801) receptor antagonists altered the acquisition and/or expression of CO-CFP. The CFP was produced by training rats to drink one novel flavor (CS+, e.g., cherry) mixed in a 3.5% CO solution and another flavor (CS–, e.g., grape) in a 0.9% CO solution. In expression studies, food-restricted rats drank these solutions in one-bottle sessions (2 h) over 10 days. Subsequent two-bottle tests with the CS+ and CS– flavors mixed in 0.9% CO solutions occurred 0.5 h after systemic administration of vehicle (VEH), NTX (0.1–5 mg/kg) or MK-801 (50–200 ug/kg). Rats displayed a robust CS+ preference following VEH treatment (85–88%) which was significantly though moderately attenuated by NTX (69–70%). The lower doses of MK-801 slightly reduced the CS+ preference; the high dose blocked the CS+ preference (49%) but also markedly reduced overall CS intake. In separate acquisition studies, rats received VEH or NTX (0.1, 0.5, 1 mg/kg) or MK-801 (100 ug/kg) 0.5 h prior to 1-bottle training trials with CS+/3.5% CO and CS–/0.9% CO training solutions. Additional Limited VEH groups were trained with intakes limited to that of the NTX and MK-801 groups. Subsequent two-bottle CS+ vs. CS– tests were conducted without injections. Significant and persistent CS+ preferences were observed in VEH (77–84%) and Limited VEH (88%) groups. NTX treatment during training failed to block the acquisition of CO-CFP although the magnitude of the CS+ preference was reduced by 0.5 (70%) and 1.0 (72%) mg/kg doses relative to the Limited VEH treatment (88%). In contrast, MK-801 (100 ug/kg) treatment during training blocked the acquisition of the CO-CFP. These data suggest a critical role for NMDA, but not opioid receptor signaling in the acquisition of a fat conditioned flavor preferences, and at best limited involvement of NMDA and opioid receptors in the expression of a previously learned preference.

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Keywords

Corn Oil; Naltrexone; MK-801; Learning

Introduction

The overconsumption of sugars and fats appear to be triggered by both their inherent hedonic properties as well as their respective abilities to trigger learned preferences (Dela Cruz et al., 2012; Sclafani, 1999; Sclafani & Ackroff, 2012). Preferences can be elicited on the basis of learned associations either between flavor cues and the orosensory aspects of sugars or fats (flavor–flavor conditioning), or between flavor cues and the nutrient’s postingestive consequences (flavor–nutrient conditioning). The brain dopamine (DA) system has been identified as one neurochemical substrate of conditioned flavor preferences (CFP) elicited by sugars (Azzara et al., 2001; Baker et al., 2003; Hsiao and Smith, 1995; Yu et al., 2000a, 2000b). Flavor-flavor conditioning studies used rats that were either sham-fed sucrose (Yu et al., 2000a, 2000b) or real-fed fructose (Baker et al., 2003), and identified a critical role for DA D1 and D2 receptor signaling in the acquisition and expression of a sweet taste-induced CFP. In contrast, flavor-nutrient conditioning studies involving intragastric (IG) sucrose infusions (Azzara et al., 2000), identified a critical role for DA D1, but not D2 receptor signaling in the acquisition, and to a lesser degree, the expression of post-oral sugar-induced CFP. Brain sites involved in DA modulation of sugar-CFP include the nucleus accumbens NAc: Bernal et al., 2008; Touzani et al., 2008), amygdala (AMY: Bernal et al., 2009; Touzani et al., 2009) and medial prefrontal cortex (mPFC: Malkusz et al., 2012; Touzani et al., 2010). NMDA receptor signaling was also implicated in the acquisition, but not the expression of fructose-CFP given the ability of the non-competitive NMDA antagonist, MK-801 to block this response (Golden and Houpt, 2007). In contrast, systemic or central administration of the general opioid antagonist, naltrexone (NTX) had little or no effect on the flavor preferences conditioned by the sweet taste or post-oral actions of sugar (Azzara et al., 2000; Baker et al., 2004; Bernal et al., 2010; Yu et al., 1999).

Rodents are also attracted to the flavor of fat (e.g., corn oil, CO) from an early age, which may be mediated in part by taste receptors for fatty acids and trigeminal “mouth feel” receptors (Ackroff & Sclafani, 2009). In addition, both the postingestive actions and orosensory properties of fat are rewarding and condition a flavor preference (Sclafani, 1999; Ackroff & Sclafani, 2009). However, limited DA D1 and D2 antagonist involvement was observed for the acquisition and expression of CO-CFP (Dela Cruz et al., 2012). The present study expanded the analysis of potential neurochemical substrates mediating CO-CFP by examining the opioid and NMDA signaling systems.

The opioid system has been implicated in the mediation of fat appetite and intake. In particular, there are many reports of opioid receptor antagonists suppressing fat intake in rats and mice (Cole et al., 1995; Dym et al., 2010; Glass et al., 2000; Higgs and Cooper, 1998; Islam and Bodnar, 1990; Marks-Kaufman et al., 1985; Naleid et al., 2007; Sahr et al., 2008). In addition, administration of the mu-selective opioid agonist, DAMGO into the NAC stimulated high-fat intake in rats (Zhang et al., 1998). Place preferences conditioned by oral intake of corn oil or a high-fat snack food are also attenuated by naltrexone administration (Jarosz et al., 2006; Shide and Blass, 1991). However, the role of opioid receptors in fat-conditioned flavor preferences has not been investigated.

Glutamate signaling has been shown to play a crucial role in learning and memory and the underlying synaptic plasticity (Rezvani, 2006). More specifically, glutamate receptor activation is required for food-related incentive learning. That is, glutamate antagonism

within the AMY and NAc impaired appetitive instrumental learning (Kelley et al., 1997; Hernandez et al., 2005). Glutamate antagonism within the AMY also impaired both the acquisition and expression of conditioned taste avoidance (Yasoshima et al., 2000). Within the VTA, glutamate antagonists impaired cue-sucrose learning and DA release in the NAc elicited by the sucrose-predictive cue (Stuber et al., 2008; Zellner et al., 2009; Zweifel et al., 2009). Interestingly, glutamate receptor antagonism by systemic administration of MK-801 blocked the acquisition of fructose-CFP (Golden & Houpt, 2007).

Therefore, the present study examined whether systemic administration of opioid (NTX) and NMDA (MK-801) receptor antagonists would attenuate flavor preference conditioning by CO ingestion. To this end, different doses of NTX or MK-801 were injected systemically either prior to testing (expression experiments) or training (acquisition experiments) sessions. Based on the observed reductions in fat intake produced by NTX (Cole et al., 2005; Dym et al., 2010; Glass et al., 2000; Higgs and Cooper, 1998; Islam and Bodnar, 1990; Jarosz et al., 2006; Marks-Kaufman et al., 1985; Naleid et al., 2007; Sahr et al., 2008; Zhang et al., 1998), we expected that opioid receptor antagonism would impair both the acquisition and expression of CO-CFP. Based upon the ability of MK-801 to block the acquisition, but not the expression of fructose-CFP (Golden and Houpt, 2007) and its other effects on food learning (Hernandez et al., 2005; Kelley et al., 1997; Stuber et al., 2008; Yasoshima et al., 2000; Zellner et al., 2009; Zweifel et al., 2009), we expected that NMDA receptor antagonism would impair the acquisition, but not the expression of CO-CFP.

Methods

Subjects

Male Sprague-Dawley rats (260–300 g, Charles River Laboratories, Wilmington, MA) were housed individually in wire mesh cages and maintained on a 12:12 h light/dark cycle with chow (5001, PMI Nutrition International, Brentwood, MO) and water available *ad libitum*, except as noted below. The experimental protocols were approved by the Queens College Institutional Animal Care and Use Committee certifying that all subjects and procedures are in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Test Solutions

The training fluids consisted of 3.5% and 0.9% corn oil (CO: Sigma Chemical Co., St. Louis, MO) flavored with 0.05% unsweetened grape or cherry Kool-Aid (General Foods, White Plains, NY) and prepared as suspensions using 0.3% xanthan gum (Sigma) as used in our prior study (Dela Cruz et al., 2012). Half of the rats in each group had the cherry flavor added to the 3.5% CO and the grape flavor added to the 0.9% CO; the flavors were reversed for the remaining rats. In the two-bottle preference tests, the cherry and grape flavors were each presented in 0.9% CO. The CO + Kool-Aid + gum mixtures are hereafter referred to as solutions. The flavored training solutions are referred to as the CS+/3.5% CO and CS-/0.9% CO. The flavored 0.9% solutions used in the two bottle tests are referred to as CS+ and CS-. All testing took place in the rat's home cage during the mid-light phase of the light:dark cycle. In the two weeks prior to testing, the rats were placed on a food restriction schedule that maintained their body weights at 85–90% of their *ad libitum* level. The rats were initially adapted to drink an unflavored 0.2% saccharin solution from sipper tubes during daily 2-h sessions. The sipper tube was mounted on the front of the cage held by a taut steel spring, and was positioned 3–6 cm above the cage floor. This training procedure was repeated daily until all rats approached the sipper tubes with short (< 1 min) latency, typically within three days. The limited food rations were given 30 min after each training session.

Experiment 1: NTX and CO-CFP: Expression Study

Eleven male rats were given ten 1-bottle training sessions (2 h/day) with 24 ml of the CS+/3.5% CO solution presented on odd-numbered days, and 24 ml of the CS-/0.9% CO solution presented on even-numbered days. On days 9 and 10, the rats had access to a second sipper tube containing water. This familiarized the rats to the presence of two sipper tubes used during the choice tests; water intake was negligible in these training trials. The left-right position of the CS and water sipper tubes was counterbalanced over the two days. Following training, all rats were given ten daily two-bottle choice test sessions (2 h/day) with the CS+ and CS- solutions. Thirty min prior to the first two sessions, all rats were given vehicle injections (1 ml 0.9% saline/kg body weight, sc). Then the rats received sc treatment with four doses (0.1, 0.5, 1 and 5 mg/kg) of NTX (Sigma Chemical Co., St. Louis, MO) prior to the remaining sessions; half of the rats were tested with an ascending dose order, and the remaining rats were tested with a descending dose order. The rats were tested in two consecutive daily sessions at each drug dose with the left-right position of the CS+ and CS- solutions counterbalanced across sessions to control for side effects. The antagonist dose range was identical to that used in our prior conditioning studies with sugars (Azzara et al., 2000; Baker et al., 2004; Yu et al., 1999). Care was taken to minimize spillage due to the fact that some of the effects could be potentially small. After initially weighing each bottle, it was gently shaken to insure appropriate flow of the viscous corn oil solutions. Any effluent from the bottle (~ 0.5–1.0 g) was collected and appropriate spillage adjustments were made to obtain an accurate pre-weight measurement. The sipper tube was occluded when the bottles were placed onto the cage and subsequently removed. The taut steel spring prevented movement of the bottles during the sessions. Visual inspection of the bottles during the study revealed minimal if any spillage because of the viscosity of the solutions. The session length of 2 h was identical to that previously used in assessing fructose-CFP (Baker et al., 2003, 2004), and CO-CFP (Dela Cruz et al., 2012).

Experiment 2: NTX and CO-CFP: Acquisition Study

Five groups of naïve male rats were matched for their intakes of an unflavored 0.2% saccharin solution prior to training. The rats were given ten 1-bottle training sessions (2 h/day, 24 ml) with the CS+/3.5% CO solution presented on odd-numbered sessions, and the CS-/0.9% CO solution presented on even-numbered sessions. The first group (VEH, n=8) received vehicle (1 ml 0.9% saline/kg body weight, sc) injections 30 min prior to each training session. The second (NTX0.1, n=8), third (NTX0.5, n=10) and fourth (NTX1.0, n=10) groups received daily sc injections of NTX at respective doses of 0.1, 0.5 and 1 mg/kg 30 min prior to each training session. Because NTX reduced overall CS intakes, the fifth group (Limited VEH) of 17 rats received daily sc injections of vehicle 30 min prior to each training session, and their intakes were limited to approximate the reduced intakes observed in the different drug dose groups. These doses were similar to those employed in acquisition studies with sugars (Baker et al., 2004; Yu et al., 1999). Following training, all five groups were given six daily two-bottle choice sessions (2 h/day) with unlimited access to the CS+ and CS- solutions; no drugs were administered prior to these sessions. The positions of the CS+ and CS- solutions were counterbalanced across sessions.

Experiment 3: MK-801 and CO-CFP: Expression Study

Fourteen naïve male rats received identical expression training and testing procedures described above except that vehicle (i.p. injections) and three doses (50, 100, 200 ug/kg, i.p.) of MK-801 (Sigma) was administered prior to two-bottle test sessions. This dose range bracketed the 100 ug/kg MK-801 dose used in a previous study evaluating expression of fructose-CFP (Golden and Houtp, 2007).

Experiment 4: MK-801 and CO-CFP: Acquisition Study

Three groups of naïve male rats received identical acquisition training and testing procedures described above except that vehicle (VEH, $n=8$, i.p.), MK-801 (MK100, $n=12$, 100 $\mu\text{g/kg}$, i.p.) and a Limited VEH ($n=10$) condition were administered during the ten one-bottle training sessions. Because MK-801 reduced overall CS intakes, this last group was necessary to approximate the reduced intake. The MK-801 dose was identical to that employed in an acquisition study with fructose (Golden and Houpt, 2007).

Data analysis

In the expression studies, training intakes were averaged over the five CS+/3.5% CO and five CS-/0.9% CO sessions and evaluated by t-tests. Intakes during the preference tests were averaged over the two sessions at each dose and evaluated with two-way repeated-measures analyses of variance (ANOVA, CS condition vs. Dose) for the NTX and MK-801 groups, respectively. Separate ANOVAs evaluated percent CS+ intakes and total intakes as a function of dose for the two groups.

In the two acquisition studies, training intakes were averaged over the five CS+/3.5% CO and CS-/0.9% CO sessions and were analyzed separately in a two-way randomized-blocks ANOVA (CS x Groups). Intakes during the preference tests were averaged over sessions 1–2, 3–4, and 5–6 (referred to as Tests 1, 2, and 3) to control for side position effects. A three-way randomized-blocks ANOVA compared the CS intakes of the NTX and control groups and of the MK-801 and control groups (Group x CS x Test). Separate two-way ANOVAs evaluated percent CS+ intakes and total intakes of the groups. When main or interaction effects were found, Bonferroni corrected comparisons ($p<0.05$) detected significant effects.

Results

Experiment 1. Opioid receptor antagonism and expression of CO-CFP

The mean 1-bottle training intakes of the CS+/3.5% CO (22.0 ± 0.4 g/2 h) was significantly greater ($t(10) = 6.12$, $p<0.0001$) than the CS-/0.9% CO (16.0 ± 1.2 g/2 h). In the two-bottle choice tests, overall, rats consumed significantly more CS+ than CS- ($F(1,50) = 120.98$, $p<0.0001$); total intake significantly varied as a function of drug dose ($F(4,50) = 4.68$, $p<0.028$), and there was a significant CS x Dose interaction ($F(4,50) = 4.03$, $p<0.0065$). CS+ intakes significantly exceeded CS- intakes following vehicle and all NTX doses (Figure 1). Rats consumed significantly less CS+ at all NTX doses compared to vehicle, whereas CS- intakes failed to be significantly affected (Figure 1). Total intake (g/2 h) significantly declined following the 0.1 (28.7 ± 2.4 g), 0.5 (24.7 ± 1.5 g), 1 (24.0 ± 2.4 g) and 5 (26.0 ± 1.9 g) mg/kg NTX doses relative to vehicle (33.6 ± 1.3 g). Significant differences in the percent CS+ intakes were observed ($F(4,40) = 3.35$, $p<0.019$), and the preferences at the 0.1 (69%) and 1 (71%) mg/kg NTX doses were significantly lower than the preference (88%) following vehicle (Figure 1). Preferences at the 0.5 (77%) and 5 (74%) mg/kg NTX doses were intermediate, but failed to significantly differ from the vehicle test.

Experiment 2. Opioid receptor antagonism and acquisition of CO-CFP

During 1-bottle training, overall CS+/3.5% CO intake (14.8 g/2 h) significantly ($F(1,64) = 113.11$, $p<0.0001$) exceeded CS-/0.9% CO intake (8.8 g/2 h), and significant differences were observed among the five groups ($F(4,64) = 30.11$, $p<0.0001$) but not for the Group x CS interaction ($F(4,64) = 0.97$). Total training intakes (2 h/g) were significantly greater in the VEH group (18.1 g) than the Limited VEH (10.3 g), NTX0.5 (10.3 g) and NTX1.0 (5.7 g) groups; intakes of the later three groups did not differ. CS+/3.5% CO training intake was significantly greater than CS-/0.9% CO intake in all groups (Figure 2A). CS+/3.5% CO and CS-/0.9% intakes of the NTX0.5, NTX1.0 and Limited VEH groups were significantly

lower than the corresponding VEH animals. However, CS+/3.5% CO and CS-/0.9% CO intakes of the NTX0.5 and NTX1.0 groups failed to differ from those of the Limited VEH group except for lower intake in the CS-0.9% CO NTX1.0 group (Figure 2A).

In the two-bottle preference tests, overall, rats consumed significantly more ($F(1,128)=752.95$, $p<0.0001$) CS+ (25.0 g/2 h) than CS- (6.6 g/2 h) solution, and significant differences were observed among the five groups ($F(4,64)=17.69$, $p<0.0001$), among tests ($F(2,32)=23.95$, $p<0.0001$) and for the interactions between Groups x Tests ($F(8,128)=16.15$, $p<0.001$), Groups x CS ($F(4,64)=11.49$, $p<0.0001$), and among Groups, Tests and CS ($F(8,128)=4.31$, $p<0.0001$). Total CS intake was significantly lower in the NTX1.0 group (18.0 g) relative to VEH (38.6 g) or Limited VEH (32.0 g) groups during testing; the NTX0.1 and NTX0.5 groups were similar to the control groups. CS+ intakes significantly exceeded CS- intakes across all three tests in VEH (Figure 2B), Limited VEH (Figure 2F), NTX0.1 (Figure 2C) and NTX0.5 groups (Figure 2D). In the NTX1.0 group, CS+ intakes significantly exceeded CS- intakes in Tests 2 and 3, but not in Test 1 (Figure 2E). The NTX1.0 group displayed significantly less CS+ intake in all three tests relative to the VEH and Limited VEH groups. The NTX0.5 group displayed significantly less CS+ intake in Test 1 than the VEH and Limited VEH groups, and significantly more CS- intake in Tests 2 and 3 than the Limited VEH group (Figure 2).

Significant differences in the percent CS+ intakes were observed among groups ($F(4,64)=8.49$, $p<0.0001$), but not among tests ($F(2,32)=1.30$), or for the interaction between Groups x Tests ($F(8,128)=1.76$). An analysis of the percent CS+ intakes averaged over the three tests revealed that the percent CS+ intakes of the NTX0.5 (70%,) and NTX1.0 (72%) groups were significantly lower than that of the Limited VEH group (88%) but not the VEH (77%) group. The NTX0.1 (89%), VEH and Limited VEH groups did not significantly differ in their CS+ preferences.

Experiment 3. NMDA receptor antagonism and expression of CO-CFP

The mean 1-bottle training intake of the CS+/3.5% CO (20.1 ± 0.8 g/2 h) failed to differ significantly from intake of the CS-/0.9% CO (18.4 ± 0.7 g/2 h). In the two-bottle preference tests, overall, the rats consumed significantly more CS+ than CS- ($F(1,52)=53.81$, $p<0.0001$); total intake significantly varied as a function of drug dose ($F(3,52)=25.08$, $p<0.001$), and there was a significant CS x Dose interaction ($F(3,52)=6.71$, $p<0.0007$). CS+ intakes significantly exceeded CS- intakes at the 0 (vehicle), 50 and 100, but not at the 200 ug/kg doses of MK-801 (Figure 3). Rats consumed significantly less CS+ at the 200 ug/kg MK-801 dose as compared to vehicle, whereas CS- intakes failed to be significantly affected (Figure 3). Total intake (g/2 h) failed to differ among the 0 (30.0 ± 2.8 g), 50 (28.5 ± 2.8 g) and 100 (20.4 ± 3.2 g) ug/kg MK-801 doses, but was significantly lower at the 200 (2.0 ± 1.1 g) ug/kg MK-801 dose. Significant differences in the percent CS+ intakes were observed ($F(3,39)=4.71$, $p<0.007$), and the preference (49%) at the 200 ug/kg MK-801 dose was significantly lower than the preference (85%) following vehicle (Figure 2). Preferences at the 50 (72%) and 100 (70%) ug/kg MK-801 doses were intermediate, but failed to significantly differ from the vehicle test.

Experiment 4. NMDA receptor antagonism and acquisition of CO-CFP

During 1-bottle training, overall CS+/3.5% CO intake (14.8 g/2 h) significantly ($F(1,22)=101.17$, $p<0.0001$) exceeded CS-/0.9% CO intake (8.6 g/2 h), and significant differences were observed among the three groups ($F(2,22)=15.79$, $p<0.0001$) but not for the Group x CS interaction ($F(2,22)=0.22$). Total training intake of the VEH group (33.0 g) was significantly greater than that of the Limited VEH (19.6 g) and MK100 (17.8 g) groups. CS

+3.5% CO training intake was significantly greater than CS-/0.9% CO intake in all groups (Figure 4A).

In the two-bottle preference tests, overall the rats consumed significantly more ($F(1,44)=108.97$, $p<0.0001$) CS+ (20.8 g) than CS- (5.7 g) solution, and significant differences were observed among the three groups ($F(2,22)=6.29$, $p<0.007$) and for the interactions between Groups x Tests ($F(4,44)=9.55$, $p<0.01$) and Groups x CS ($F(2,22)=26.36$, $p<0.0001$), but not among tests or the other interactions. Total CS intake was significantly lower in the MK100 group (19.7 g/2 h) relative to VEH (28.2 g/2 h) or Limited VEH (31.6 g/2 h) groups. CS+ intakes significantly exceeded CS- intakes across all three tests in the VEH (Figure 4B) and Limited VEH (Figure 4D) groups. In contrast, CS+ and CS- intakes failed to differ in all three tests in the MK100 group (Figure 4C). The MK100 group displayed significantly less CS+ intake in the first two tests relative to the VEH group, and in all three tests relative to the Limited VEH group (Figure 4C).

Significant differences in the percent CS+ intakes were observed among groups ($F(2,22)=13.18$, $p<0.0002$), but not among tests ($F(2,22)=1.60$), or for Group x Test interaction ($F(4,44)=1.62$). An analysis of the percent CS+ intakes averaged over the three tests revealed that the MK100 group (63%) displayed significantly lower CS+ preferences than did the VEH (84%) and Limited VEH (88%) groups.

Discussion

The present study extended our previous findings (Dela Cruz et al., 2012) of a robust fat-CFP produced by mixing one novel flavor (CS+) in 3.5% CO and a second novel flavor (CS-) in 0.9% CO. DA D1 receptor antagonism during training with SCH23390 (200–400 nmol/kg) failed to significantly reduce the acquisition of CO-CFP, and raclopride treatment (25–200 nmol/kg) had a limited effect on CO-CFP acquisition. SCH23390 and raclopride also had limited effects on the expression of a previously learned CO-CFP and in the case of D1 antagonist, this was associated with substantial reductions in overall CS intakes. These data suggested that other neurochemical receptor systems are involved in mediating the acquisition and expression of fat-CFP, and thus the present study examined the roles of opioid and NMDA receptor systems in these responses.

Opioid Receptor Antagonism and Fat-CFP

Given the observed reductions in fat intake following general opioid antagonism (Cole et al., 2005; Dym et al., 2010; Glass et al., 2000; Higgs and Cooper, 1998; Islam and Bodnar, 1990; Jarosz et al., 2006; Marks-Kaufman et al., 1985; Naleid et al., 2007; Sahr et al., 2008; Zhang et al., 1998), we hypothesized that systemic NTX treatment would impair both the acquisition and expression of CO-CFP. In the expression study, although all doses of NTX significantly reduced CS+, but not CS- intakes, significant preferences (defined by significantly greater CS+ over CS- intake) were observed following VEH and all NTX doses. However, the magnitude of the CO-CFP was significantly, but marginally reduced at 0.1 (69%) and 1 mg/kg (71%) doses, but not at the 0.5 (77%) and 5 (74%) mg/kg doses relative to VEH treatment (88%). Thus, opioid receptor antagonism appears to be minimally involved in the expression of a preference for a fat nutrient source, yet intimately involved in the maintenance of fat intake per se.

In the acquisition study, the three training doses of NTX significantly decreased both CS+/3.5% CO and CS-/0.9% CO intakes relative to the VEH group. These opioid-induced reductions in the both corn oil concentrations are consistent with prior data (Cole et al., 2005; Dym et al., 2010; Glass et al., 2000; Higgs and Cooper, 1998; Islam and Bodnar, 1990; Jarosz et al., 2006; Marks-Kaufman et al., 1985; Naleid et al., 2007; Sahr et al., 2008;

Zhang et al., 1998). Overall, CS+ intakes significantly exceeded CS− intakes in VEH and Limited VEH control groups as well as in all NTX groups. However, the magnitude of the CS+ preference was reduced in the NTX0.5 (70%) and NTX1.0 (72%) groups relative to the Limited VEH group (88%) although not relative to the VEH group (77%). Thus, while opioid receptor antagonism does not block the acquisition of a CO-CFP, it does attenuate the strength of the learned preference to some degree. This contrasts with the failure of NTX treatment to reduce the acquisition of sugar-conditioned flavor preferences (Azzara et al., 2000; Baker et al., 2004; Yu et al., 1999), but it should be noted that different training procedures were used in the fat- and sugar-conditioning studies. In particular, whereas the rats in the present study were exposed to both the oral and post-oral actions of corn oil, the rats in the prior studies exposed to only the oral (sweet taste) or post-oral actions of sugars.

NMDA Receptor Antagonism and Fat-CFP

Given the ability of MK-801 to block the acquisition, but not the expression of fructose-CFP (Golden and Houpt, 2007), we hypothesized that NMDA receptor antagonism would have similar effects on the acquisition and expression of CO-CFP. This hypothesis was confirmed. In the expression study, MK-801 significantly and dose-dependently reduced CS+, but not CS− intakes, and significant preferences were observed following VEH (85%) and 50 (72%) and 100 ug/kg (70%) MK-801 doses. The loss of preference (49%) following the 200 ug/kg MK-801 dose was accompanied and presumably due to a dramatic decrease in total CS intake (to 2 ml/2 h); note that a 200 ug/kg dose was not used in the Golden and Houpt study (2007). Therefore, as previously observed for fructose-CFP (Golden and Houpt, 2007), NMDA receptor signaling appears to be minimally involved in the expression of a previously learned fat-based flavor preference.

In the acquisition study, treatment with 100 ug/kg MK-801 during training significantly decreased both CS+/3.5% CO and CS−/0.9% CO intakes compared to the vehicle treatment. Nevertheless, training intakes of the CS+/3.5% CO exceeded that of the CS−/0.9% CO in the MK801 group as well as in the VEH and Limited VEH groups. This demonstrates that the MK801 group discriminated between the two CS training solutions and found the CS+/3.5% CO the more attractive solution. Yet, in the CS+ vs. CS− choice tests, the MK801 group, unlike the VEH and Limited VEH groups, failed to drink more CS+ than CS−. This was due to the significant and selective reduction in CS+ intake in the MK-801 group relative to vehicle groups, an effect that was identical to the abolition of the acquisition of fructose-CFP by MK-801 (Golden and Houpt, 2007). Consistent with this finding, the percent CS+ intake of MK801 group (63%) was significantly lower than that of the VEH (84%) and Limited VEH (88%) groups. Importantly, the robust CS+ conditioning displayed by the Limited VEH group indicates that the inhibition of preference conditioning by MK-801 treatment was not secondary to the drug-induced suppression in CS training intakes. Thus, the present data along with those of Golden and Houpt (2007) indicate an important role of NMDA signaling in the acquisition of both fructose- (Golden and Houpt, 2007) and corn oil-CFP.

A role of glutamate and its receptors in learning has been well-characterized (see review: Rezvani, 2006), and has been specifically linked to food reward-related learning. NMDA receptor antagonism within the AMY and NAc impaired appetitive instrumental learning (Kelley et al., 1997; Hernandez et al., 2005). NMDA receptor antagonism within the AMY also impaired the acquisition of conditioned taste avoidance (Yasoshima et al., 2000). Within the VTA, NMDA receptor antagonism impaired cue-sucrose learning and DA release in the NAc elicited by the sucrose-predictive cue (Stuber et al., 2008; Zellner et al., 2009; Zweifel et al., 2009). Finally, glutamate also interacted with DA within the NAc, AMY and mPFC to modulate reward-related appetitive learning (Smith-Roe & Kelley, 2000; Baldwin et al., 2002; Andrzejewski et al., 2004). Future studies should investigate the

role of NMDA receptor antagonism within the NAc, AMY and mPFC on the acquisition of fructose- and CO-CFP, and any interaction it may enjoy with DA signaling

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Highlights

- Rats display robust fat-conditioned flavor preferences (CFP).
- Opioid antagonism marginally reduces the expression of fat-CFP.
- Opioid antagonism fails to alter acquisition of fat-CFP.
- NMDA antagonism reduces fat CFP expression only with overall intake suppression.
- NMDA antagonism blocks acquisition of fat-CFP.

Figure 1. (Expression Study): Intakes (mean in g/2 h +SEM, 2 h) of CS+ and CS– solutions in two-bottle preference tests in animals receiving systemic injections of the opioid antagonist, naltrexone 30 min prior to testing. Significant differences are denoted between CS+ and CS– intake within an injection condition (*) and between CS+ intake following a drug dose relative to vehicle treatment (+). The percentages of CS+ intake over total intake are denoted above each pair of values with significant differences relative to vehicle treatment (*) noted.

Corn Oil-CFP Acquisition: Opioid Receptor Antagonism

Figure 2.

(Acquisition Study): Training intakes (mean in g/2 h +SEM) of rats exposed to ten 1-bottle sessions of flavored 3.5% corn oil solutions (CS+/3.5% CO, Days 1, 3, 5, 7, 9) or 0.9% corn oil solutions (CS-/0.9% CO, Days 2, 4, 6, 8, 10) 30 min following systemic injections of vehicle (Veh), the opioid antagonist, naltrexone at doses of 0.1 (N0.1), 0.5 (N0.5) or 1 (N1) mg/kg. A fifth group (Limited) received vehicle injections and had CS+/3.5% CO and CS-/0.9% CO intakes limited to approximate the intakes of the drug groups. Significant differences are denoted between CS+/3.5% CO or CS-/0.9% CO intake following a drug dose relative to VEH (+) or LTD (#) treatment (Panel A). Intakes (mean in g/2 h +SEM) of CS+ and CS- in three two-bottle preference tests in the VEH (Panel B), NTX0.1 (Panel C), NTX0.5 (Panel D), NTX1 (Panel E) and Limited VEH (Panel F) groups. Significant differences (*) are denoted between CS+ and CS- intake within each test and each group, and between CS+ or CS- intakes following a particular drug group relative to VEH (+) or Limited VEH (#) conditions.

Figure 3. (Expression Study): Intakes (mean in g/2 h $\pm$ SEM) of CS+ and CS- solutions in two-bottle preference tests in animals receiving systemic injections of the NMDA antagonist, MK-801 30 min prior to testing. Significant differences are denoted between CS+ and CS- intake within an injection condition (*) and between CS+ intake following a drug dose relative to vehicle treatment (+). The percentages of CS+ intake over total intake are denoted above each pair of values with significant differences relative to vehicle treatment (*) noted..

Corn Oil-CFP Acquisition: NMDA Receptor Antagonism

Figure 4.

(Acquisition Study): Training intakes (mean in g/2 h $\pm$ SEM) of rats exposed to ten 1-bottle sessions of flavored 3.5% corn oil solutions (CS+/3.5% CO) or 0.9% corn oil solutions (CS-/0.9% CO) 30 min following systemic injections of vehicle (VEH) or the NMDA antagonist, MK-801 (100 ug/kg). A third group (Limited) received vehicle injections and had CS+/3.5% CO and CS-/0.9% CO intakes limited to approximate the intakes of the drug groups. Significant differences are denoted between CS+/3.5% CO or CS-/0.9% CO intake following a drug dose relative to VEH (+) or Limited (#) treatment (Panel A). Intakes (mean in g/2 h $\pm$ SEM, 2 h) of CS+ and CS- in three two-bottle preference tests in the VEH (Panel B), MK-801 (Panel C) and Limited Vehicle (Panel D) groups. Significant differences (*) are denoted between CS+ and CS- intake within each test and each group, and between CS+ or CS- intakes following a particular drug group relative to VEH (+) or Limited VEH (#) conditions.