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Glucose-conditioned flavor preference learning requires co-activation of NMDA and dopamine D1-like receptors within the amygdala

Khalid Touzani¹, Richard J. Bodnar², and Anthony Sclafani¹

¹Department of Psychology Brooklyn College of the City University of New York, 2900 Bedford Avenue, Brooklyn, NY 11210

²Department of Psychology Queens College of the City University of New York, 65-30 Kissena Boulevard, Queens, NY 11367

Abstract

The role of amygdala (AMY) NMDA receptor signaling and its interaction with dopamine D1-like receptor signaling in glucose-mediated flavor preference learning was investigated. In Experiment 1, rats were trained with a flavor (CS+) paired with intragastric (IG) 8% glucose infusions and a different flavor (CS-) paired with IG water infusions. In the two-bottle tests (Expression), bilateral intra-AMY injections of the NMDA receptor antagonist, AP5 (0, 5 and 10 nmol/brain), did not block the CS+ preference. In Experiment 2, new rats received intra-AMY injections of either vehicle or AP5 (10 nmol), prior to training sessions with CS+/IG glucose and CS-/IG water. In the two-bottle tests without drug treatment, AP5 rats failed to prefer the CS+ flavor (50%). In Experiments 3, new rats were trained as in Experiment 2 except that, during training, half the rats received AP5 injections (5 nmol) in one side of the AMY and SCH23390 (D1-like receptor antagonist, 6 nmol), in the contralateral AMY (Drug/Drug group). The remainder rats received vehicle injections in one side of the AMY and either AP5 (5 nmol) or SCH23390 (6 nmol) in the contralateral AMY (Drug/Vehicle group). The two-bottle choice tests without drug treatment revealed that, unlike the Drug/Vehicle group (85%), the Drug/Drug group failed to prefer the CS+ flavor (50%). These results reveal an essential role for AMY NMDA receptor activation in the acquisition of flavor preference learning induced by the post-oral reinforcing properties of glucose and demonstrate that such learning is based on co-activation of NMDA and DA D1 receptors within this forebrain structure.

Keywords

AP5; Conditioning; Carbohydrate; Forebrain; Learning; Rat; SCH23390

Introduction

In conditioned flavor preferences (CFP), associations readily form between a flavor cue and the oral and/or post-oral reinforcing properties of a nutrient. One particular learning process that has been extensively studied in our laboratory is referred to as flavor-nutrient learning

Corresponding author: Dr. Khalid Touzani, Department of Psychology, Brooklyn College of the City University of New York, 2900 Bedford Avenue, Brooklyn, NY 11210, USA, TEL: 718-951-5606, FAX: 718-951-4824, ktouzani@brooklyn.cuny.edu.

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by which a preference develops for a flavor cue that is paired with the post-oral positive consequences of a nutrient (Sclafani, 1995).

The neural mechanisms underlying flavor-nutrient learning are not fully understood. The amygdala (AMY) has long been implicated in motivation and reward-related learning (Cardinal, Parkinson, Hall, & Everitt, 2002; Baxter & Murray, 2002) and we have reported that large AMY lesions prevent flavor-nutrient learning, whereas lesions limited to the basolateral amygdaloid nuclei (BLA) only attenuated this learning (Touzani & Sclafani, 2005). Our study, however, did not reveal the neurochemical systems involved in the synaptic plasticity underlying this learning.

The AMY receives both dopaminergic and glutamatergic inputs (Asan, 1997; Sah, Farber, Lopez de Armentia, & Power, 2003). Furthermore, AMY neurons express both dopamine (DA) D1-like receptors and glutamate NMDA receptors (Pickel, Colago, Mania, Molosh, & Rainnie, 2006). Several lines of evidence implicated DA D1-like receptor signaling within the AMY in food reward-related learning (Heffner, Hartman, & Seiden, 1980; Harmer & Phillips, 1999; Andrzejewski, Spencer, & Kelley, 2005). We observed that flavor-nutrient learning also depends on this signaling in the AMY (Touzani, Bodnar, & Sclafani, 2009). Glutamate transmission in the AMY through NMDA receptors is also implicated in food-related learning (Baldwin, Holahan, Sadeghian, & Kelley, 2000; Andrzejewski, Sadeghian, & Kelley, 2004). In these studies, NMDA receptor antagonism by intra-AMY administration of the competitive antagonist, 2-amino-5-phosphonopentanoate (AP5), impaired the acquisition but not the expression of appetitive instrumental responses. Given the well known role of NMDA receptors in neural plasticity underlying learning and memory (Riedel, Platt, & Micheau, 2003), that NMDA and DA D1-like receptors in the AMY co-localize on the same neuron, and that synaptically-evoked NMDA currents in the AMY are modulated by DA D1-like receptors (Pickel et al., 2006), we hypothesized that DA acting on amygdalar D1-like receptors promotes flavor-nutrient learning by acting in concert with amygdalar NMDA receptors.

The present study was designed to test this hypothesis. Experiments 1 and 2 first determined the role of amygdalar NMDA receptor signaling in the acquisition and expression of glucose-CFP by injecting AP5 into the AMY prior either to training or testing sessions. These injections involved a large part of the AMY given that lesions limited to the basolateral nucleus and DA D1-like receptor antagonism in either the basolateral or central nucleus had minimal effect on flavor-nutrient learning (Touzani & Sclafani, 2005). We predicted that only CFP acquisition would be blocked by NMDA receptor antagonism in the AMY based on the differential effect of NMDA receptor antagonism in other learning paradigms (Burns, Everitt, & Robbins, 1994; Yasoshima, Morimoto, & Yamamoto, 2000; Goosens & Maren, 2004). In Experiment 3, we disrupted potential interactions between NMDA and DA D1-like receptor signaling in the AMY by injecting AP5 into one side of the AMY and SCH23390 into the other side of the AMY during the acquisition phase of a glucose-CFP. We predicted that CFP acquisition would be blocked based on our finding that D1-like receptor antagonism in the AMY prevented the acquisition of glucose-CFP (Touzani et al., 2009), and on the idea that DA interacts with glutamate postsynaptically to produce reward-related incentive learning (Wickens, 1993; Beninger, 1993).

Materials and methods

Subjects

The subjects were 71 adult male Sprague-Dawley rats obtained from Charles River Laboratories (Wilmington, MA) or bred in our laboratory; they weighed 370-480 g at the time of the surgeries. The rats were individually housed in plastic cages with stainless steel

wire lids in a vivarium maintained at 21°C and under a 12:12 h light:dark cycle (lights on at 0800h). They were maintained on chow (Laboratory Rodent Diet 5001, PMI Nutrition International, Brentwood, MO) and tap water. Experimental protocols were approved by the Brooklyn College Animal Care and Use Committee and were performed in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals.

Surgery

The rats were anesthetized with intraperitoneal injection of a ketamine hydrochloride (63 mg/Kg) and xylazine (9.4 mg/Kg) mixture and held in a Kopf stereotaxic apparatus with the incisor bar set 3.3 mm below the interaural line. Stainless steel guide-cannulae (26-gauge, i.d. = 0.24 mm; o.d. = 0.46 mm, Plastics One Inc. Roanoke, VA) were aimed at bilateral placements in the AMY using the following coordinates: 2.2 mm posterior to Bregma, 4.3 mm lateral to the sagittal suture and 8.0 mm ventral from the surface of the skull. The guide-cannulae were secured with stainless steel skull screws and dental cement. During the same brain surgery session, the rats were fitted with a gastric catheter (silastic tubing, i.d. = 1.02 mm; o.d. = 2.16 mm) that was inserted in the fundus of the stomach and secured with sutures and polypropylene mesh. The tubing was routed under the skin and connected to a connector pedestal that was mounted and secured on the animal's neck with polypropylene mesh and sutures. Intramuscular penicillin (30,000 U) was given following the surgeries. Six rats died during or few days after the surgery and one rat had one brain guide-cannula clogged.

Apparatus

As detailed in Touzani & Sclafani (2001), training and testing occurred in plastic cages that gave the rat access to one or two stainless steel drinking spouts. The spouts were attached to drinking bottles mounted on motorized holders that positioned the spouts at the front of the cage at the start of the sessions and retracted them at the end of the sessions. Licking behavior was monitored by an electronic lickometer interfaced to a microcomputer that activated a syringe pump as the rat drank. Plastic tubing connected the pump to the rat's gastric catheter through the neck-mount connector. The infusion rate was 1.3 ml/min and the ratio of oral intake and infusion volume was maintained at approximately 1:1 by the computer unless otherwise indicated.

Test solutions

The conditioned stimuli were cherry- or grape-flavored (0.05% Kool-Aid, General Foods, White Plains, NY) saccharin (0.2% sodium saccharin, Sigma-Aldrich, St. Louis, MO) solutions. Naïve rats show no preference for one of these flavors over the other. The CS+ flavor was paired with IG infusions of 8% glucose (Tate & Lyle, Honeyville Foods, Rancho Cucamonga, CA) and the CS- flavor was paired with IG water infusions. The specific flavor-infusion pairs were counterbalanced across subjects so that half the rats had cherry flavor as the CS+ and grape flavor as CS-; the flavor assignments were reversed for the remaining rats. All solutions were prepared with tap water.

Drugs and Infusion Procedures

The glutamate NMDA receptor antagonist, AP5 (Sigma-Aldrich) and the DA D1-like receptor antagonist, SCH23390 (Sigma-Aldrich) were dissolved in sterile isotonic saline (vehicle) and administered bilaterally at a volume of 0.5 µl/side. Infusions of the drugs or the vehicle into the AMY were performed bilaterally using an infusion pump (Razel Scientific Instruments, Inc., Stamford, CT) and a 33-gauge (i.d. = 0.10 mm; o.d. = 0.20 mm) stainless steel internal cannula (Plastics One) connected to a 2-µl Hamilton microsyringe (Hamilton Company, Reno, Nevada) by polyethylene tubing. During intracerebral

injections, the rats were held gently, the stylus was removed and the injection cannulae were inserted. The tip of the injection cannulae protruded 1.0 mm beyond that of the guide. The injection rate was 0.5 μ l/min and the injection cannulae were left in place an additional minute before their removal to minimize drug efflux.

Procedures

Prior to the surgery, the rats were familiarized with unflavored 0.2% saccharin solution by giving them ad libitum access to the saccharin solution along with water and chow in their home cages for three days. Then the rats were housed in the test cages overnight with ad lib access to 0.2% saccharin solution, water and food to adapt them to the test cages. The saccharin and water bottles were automatically positioned to the front of the cages for 30 min every hour. Two to three weeks after the surgery, the rats were placed on a food restriction schedule and maintained at 85% of their ad libitum body weights. They were adapted to drink the saccharin solution in the test cages during 8-10 daily 30-min sessions. During the last four sessions, the rats were connected to the infusion system and were given IG water infusions as they drank the saccharin solution.

In Experiment 1, the rats ($n=14$) were given eight one-bottle training sessions (30 min/day). In sessions 1, 3, 5 and 7, intake of the CS+ solution was paired with concurrent IG infusions of 8% glucose; in sessions 2, 4, 6, and 8, intake of the CS- solution was paired with concurrent IG infusion of water. A second drinking tube filled with water was introduced in sessions 7 and 8 to adapt the rats to the two-bottle choice procedure. The right-left positions of the CS solutions were varied using an ABBA sequence. Following training, the rats were given a series of two-bottle tests with the CS+ vs. CS- solutions with no IG infusions. The rats received bilateral injections of 0 (saline), 5 or 10 nmol of AP5 (0, 2.5 and 5 nmol/0.5 μ l/side) in the AMY, 10 min prior to the two-bottle tests (six 30 min/day sessions). The left-right position of the CS solutions alternated daily, and the rats were injected twice with each drug dose to control for side preferences. Following each 2-day block of two-bottle tests, there was a 1-day break. Half of the rats received drug injections in an ascending order and the other half in a descending order. Thus the rats received a total of four drug injections and two saline injections during testing. These injections involve a large portion of the AMY given that lesions limited to the basolateral nucleus and DA D1-like receptor antagonism in either the basolateral or central nucleus only attenuated flavor-nutrient learning (Touzani & Sclafani, 2005; Touzani et al., 2009).

In Experiment 2, the rats ($n=28$) were divided into two groups matched for their pretraining saccharin intakes. The Control group received bilateral injections of vehicle while the AP5 group received injections of a total dose of 10 nmol of AP5 (5 nmol/0.5 μ l/side) into the AMY 10 min prior to each of the CS+ and CS- training sessions (for a total of 8 injections). In sessions 1, 3, 5 and 7, intake of the CS+ solution was paired with 8 ml IG infusions of 8% glucose; in sessions 2, 4, 6, and 8, intake of the CS- solution was paired with 8 ml IG infusion of water. The IG infusions started after the rat emitted 20 licks, and continuous infusions were triggered once the rat reached 300 licks to deliver a fixed volume of 8 ml. Following each pair of training sessions with CS+ and CS- solutions, there was a 1-day break. The CS+ and CS- intakes of the Control rats were limited each day to the mean intakes of the AP5 rats, which had unrestricted access to the solutions. Following training, two-bottle preference tests (four 30 min/day sessions) were conducted during which there were no brain injections or IG infusions, and CS intakes were unlimited.

In Experiment 3 ($n=22$), the rats were trained with the same procedure used in Experiment 2 except that during training, the experimental group (Drug/Drug, $n=12$) received injections of AP5 (5 nmol/0.5 μ l) in one AMY and SCH23390 (6 nmol/0.5 μ l) in the other AMY in a counterbalanced way. The rats in the control group (Drug/Vehicle) received counterbalanced

injections of saline in one AMY and in one subgroup (n=5) AP5 (5 nmol/0.5µl) in the contralateral AMY and for another subgroup (n=5) SCH23390 (6 nmol/0.5 µl) in the contralateral AMY.

Statistical analysis

CS intakes were measured to the nearest 0.1 g and the data were analyzed using standard analyses of variance (ANOVA) procedures. Oral intakes during training and preference testing were averaged over 2- or 4-day blocks. Individual comparisons were evaluated using simple main effects tests or *t*-test when appropriate. Two-bottle preference data were also expressed as percent CS+ intake [(CS+ intake / total intake) x100]. The data were analyzed with ANOVA or *t*-test after an arcsine transformation as recommended by Kirk (1995).

Histological analysis

At the completion of the experiments, the rats were deeply anesthetized and perfused transcardially with physiological saline followed by a 10% formalin solution. The brains were removed and soaked in a 10% formalin solution containing 20% sucrose for 3-6 days. The brains were coronally sectioned with a freezing microtome at 40 µm, and the sections were mounted on gelatin-coated glass slides and stained with thionin. The positions of the cannula tracks were examined under a light microscope and reconstructed on the appropriate frontal planes of the atlas of Paxinos and Watson (1998).

Results

Experiment 1. Effects of NMDA receptor antagonism in AMY on the expression of glucose-CFP

Histology—Cannula tip placements in the AMY were deemed appropriate for ten rats (Fig. 1A), and were primarily restricted to the portion between the Frontal Planes -2.12 and -2.56 mm of the Paxinos and Watson (1998) atlas. The remaining four rats that were discarded had very rostral cannula placements between Frontal Planes -1.6 and -1.8 mm sparing most of the AMY. A photomicrograph illustrating a representative brain coronal section with injection cannula tracks positioned in the AMY is shown in Fig. 1B. There was no damage within the AMY caused by the injections and only a minor damage has been observed along the cannula track from repeated insertion.

Behavior—During one-bottle training, the intakes of the CS+ and CS- (4-day mean) did not significantly differ (9.6 and 8.8 g/30 min, respectively). In the two-bottle preference tests (Figure 2), overall, the rats consumed more CS+ than CS- [$F(1,9) = 31.6$, $p < 0.001$] and there were no Dose or CS x Dose interaction effects. Analysis of the percent CS+ intakes revealed that CS preferences did not vary as a function of AP5 dose. Thus, AP5 treatment during testing did not significantly reduce CS+ preference or total CS intakes.

Experiment 2. Effects of NMDA receptor antagonism in AMY on the acquisition of glucose-CFP

Histology—In order to have data comparable with our previous study on the effect of DA D1-like receptor antagonism on flavor-nutrient learning (Touzani et al., 2009), we included rats only with cannula tips localized in the AMY between frontal planes -2.12 and -2.56 mm of the Paxinos and Watson (1998) atlas. Placements were deemed appropriate for nine Control rats and eight AP5 rats (Fig. 3). The remaining eleven rats (6 AP5 and 5 Control) had cannula placements in the far rostral central nucleus of AMY between Frontal Planes -1.6 and -1.8 mm. Excluding these animals did not change the statistical analysis of the behavioral data.

Behavior—The rats treated with a total dose of 10 nmol (5 nmol/0.5 μ l/side) AP5 during one-bottle training consumed 9.3 g of the CS+ and 7.8 g of the CS- (4-day means) and the intakes of the Control group were limited to these amounts. There was no difference in the CS intake between the two groups and both groups consumed more CS+ than CS- [$F(1,15) = 6.82$, $p < 0.05$]. The results of the two-bottle choice tests (4-day mean) are summarized in Figure 4. The AP5 and Control groups did not significantly differ in their total CS intakes but there was a significant Group $\times$ CS interaction [$F(1,15) = 20.90$, $p < 0.001$]. Individual comparisons revealed that the Control group consumed more CS+ than CS- ($p < 0.001$) whereas the AP5 group did not differ in its intake of the CS+ and CS-solutions. The AP5 rats consumed more ($p < 0.05$) CS- and less ($p < 0.01$) CS+ than did the Control rats. Consequently, the percent CS+ intake of the Control group exceeded that of the AP5 group (75% vs. 50%, $t(15) = 4.66$, $p < 0.001$). An ANOVA of the CS intakes of the Control group and the six AP5 rats with misplaced injection cannulae revealed that the two groups did not significantly differ in their CS intakes or percent CS+ preferences (Control group: 75%; Six AP5 rats with misplaced cannulae: 63%) during the two-bottle choice tests. Thus, discrete antagonism of amygdalar NMDA receptors has no effect on flavor-nutrient learning. Thus, the 10 nmol dose of AP5 that did not impair the expression of a previously acquired glucose-CFP in Experiment 1 totally blocked the acquisition of this preference when administered during training.

Experiment 3. Effects of unilateral NMDA receptor antagonism combined with contralateral DA D1-like receptor antagonism in AMY on the acquisition of glucose-CFP

Histology—Cannula tip placements in the AMY (Fig. 5) were deemed appropriate for six Drug/Vehicle rats (three in each subgroup) and nine Drug/Drug rats and were found between frontal planes -2.12 and -2.56 mm of the Paxinos and Watson (1998) atlas. The remaining seven rats (3 Drug/Drug and 4 drug/vehicle) had misplaced cannula tips that were either in the far rostral ($n=5$) or the far caudal ($n=2$) AMY and were discarded. Excluding these animals did not change the statistical analysis of the behavioral data.

Behavior—The behavioral data from the two control subgroups did not differ so that only the combined group data are presented (AP5/Vehicle subgroup: CS+ 11.1 g, CS- 1.7 g, %CS+ 87%; SCH/Vehicle subgroup: CS+ 14.6 g, CS- 1.5 g, %CS+ 91%). During one-bottle training, the Drug/Drug rats treated with AP5 in one AMY and SCH23390 in the contralateral AMY consumed 4.1 g of the CS+ and 3.7g of the CS- (4-day means) and the intakes of the Drug/Vehicle group were limited to this amount. Both groups consumed more CS+ than CS- [$F(1,13) = 5.71$, $p < 0.05$]. This low CS intakes compared to the CS intakes of AP5 rats in Experiment 2 is mainly due to the suppressive effect of SCH23390 on fluid intake during training. The results of the two-bottle preference tests are summarized in Figure 6. The two groups did not significantly differ in their overall CS intakes but there was a significant Group $\times$ CS interaction [$F(1,13) = 8.44$, $p < 0.05$]. Individual comparisons revealed that the Drug/Vehicle group consumed more CS+ than CS- ($p < 0.01$) whereas the Drug/Drug group did not differ in its intake of the CS+ and CS- solutions. The Drug/Drug rats consumed more ($p < 0.05$) CS- and less ($p < 0.05$) CS+ than did the Drug/Vehicle rats. Consequently, the percent CS+ intake of the Drug/Vehicle group exceeded that of the Drug/Drug group (89% vs. 50%, $t(13) = 4.36$, $p < 0.001$). Thus, antagonism of NMDA receptors in one side of AMY with 5 nmol of AP5 combined with antagonism of DA D1-like receptors in the other side of AMY with 6 nmol of SCH23390 totally blocked the acquisition of a glucose-CFP.

DISCUSSION

In this study, we explored the role of AMY glutamate NMDA receptors in flavor preference learning induced by the postoral actions of glucose and the idea that acquisition of glucose-CFP requires interaction of signaling at NMDA and DA D1 receptors within this structure. The results revealed that bilateral antagonism of NMDA receptors with AP5 (5 nmol/side) during training prevented the acquisition of glucose-CFP although the same drug dose during testing did not affect the expression of a previously acquired flavor preference. Furthermore, the unilateral AMY antagonism of NMDA receptors (AP5; 5 nmol) combined with the contralateral AMY antagonism of DA D1-like receptors (SCH23390; 6 nmol) during training led to the same result, i.e., a total blockade of the acquisition of glucose-CFP. These findings add to the extensive literature on the importance of AMY NMDA receptors to learning and provide the first evidence that co-activation of DA D1-like receptors and NMDA receptors within this forebrain structure is necessary to promote flavor-nutrient preference conditioning.

Amygdala NMDA receptors and flavor-nutrient preference learning

In Experiment 1, the rats were trained to consume a CS+ flavor paired with IG infusions of 8% glucose and a CS- flavor paired with IG water infusions. In subsequent two-bottle choice tests, the rats exhibited strong CS+ preferences (88-91%) following vehicle and AP5 treatments (5-10 nmol). In Experiment 2, the 10 nmol dose of AP5 (5 nmol/side) that failed to impair the expression of a previously learned flavor preference totally blocked the acquisition of the CFP when the drug was administered in the AMY during one-bottle training. Note that the CS intakes of the Control rats were matched to those of the AP5 rats and all rats were infused with the same amount of glucose during training so that both groups were equally exposed to the CS+ flavor and the US (IG glucose). These results along with our previous lesion study (Touzani & Sclafani, 2005) indicate that the integrity of the AMY and its glutamate signaling through NMDA receptors is required for flavor-nutrient learning.

In the present study, microinjections were made in a large part of the AMY that encompasses both the BLA and CeA based on our previous observations that discrete AMY lesions limited to the BLA and DA D1-like receptor antagonism in either the BLA or CeA only attenuated flavor-nutrient learning, while much larger lesions and DA D1-like receptor antagonism involving almost the whole AMY prevented this learning (Touzani & Sclafani, 2005; Touzani et al., 2009). This outcome stands in clear contrast with the well-known role of BLA in other forms of learning such as fear conditioning and suggests that flavor-nutrient learning is a distributive function of the AMY. In fact, a more recent paper showed that both the CeA and the BLA are simultaneously recruited during tone-food pairings in an appetitive associative learning (Cole, Powell & Petrovich, 2013). However, one major feature of flavor-nutrient learning is the interoceptive (viscerosensory) nature of the nutrient US that contrasts with the exteroceptive (somatic) US used in the other forms of AMY-based learning such as electrical foot-shock. In fact, flavor-toxin learning, which also involves an interoceptive US such as intraperitoneal injection of lithium chloride, is prevented by large AMY lesions (Burt & Smotherman, 1980; Kesner et al., 1992) while lesions limited to the BLA had only minimal effects (Wang et al., 2006).

The CS flavors used in this study were mixtures of saccharin (sweet taste) and Kool-Aid flavors that are distinguishable primarily by their odors. The failure of the AP5 group to develop a significant CS+ preference cannot be attributed to an inability to discriminate the two flavors (CS+ and CS-) as a result of a drug-induced impairment in olfactory processing since the rats treated with AP5 prior to the two-bottle choice tests in Experiment 1 exhibited

a strong flavor preference. Hatfield & Gallagher (1995) also reported evidence that AP5 in the AMY does not impair olfactory discrimination.

Evidence from our laboratory indicates that pairing a saccharin-sweetened CS+ flavor with IG carbohydrate infusions enhances both the hedonic and incentive values of the flavor (Myers & Sclafani, 2001; Sclafani & Ackroff, 2006). The failure of AP5 rats to learn a glucose-CFP in Experiment 2 suggests that NMDA receptor antagonism in the AMY prevents the CS+ flavor from acquiring this enhanced reward value. It is unlikely that this outcome resulted from a blockade of the reinforcing properties of IG glucose by AP5 treatment because total lesions of AMY that prevented conditioning of a CS+ Kool-Aid flavor preference by IG carbohydrate infusions did not block conditioning of a preference for a compound taste CS+ (e.g., bitter-sweet, (Touzani & Sclafani, 2005). This suggests that the perception and processing of the nutrient US produced by IG glucose infusions was preserved after AP5 administration in the AMY.

Collectively, these findings are in line with the well-established role of NMDA glutamate receptor activation in other forms of amygdala-dependent learning, and show that activation of NMDA receptors within the AMY is critical for the acquisition, but not expression, of flavor-nutrient preference learning. Similar differential effects of NMDA antagonism within the AMY on the acquisition and expression of learned behaviors have been reported using different paradigms such as conditioned taste avoidance, discriminated approach to sucrose solution, fear conditioning and inhibitory avoidance learning (Burns et al., 1994; Yasoshima et al., 2000; Goossens & Maren, 2004). Interestingly, the induction of long-term potentiation (LTP) in the AMY is blocked by application of AP5 (Maren, 1999; Sigurdsson, Doyère, Cain, & LeDoux, 2007). Given the well-known role of NMDA signaling in neuronal plasticity underlying learning and memory, these findings indicate that a form of neural plasticity within the AMY that depends on the activation of NMDA receptors mediates the acquisition of learned responses but is not necessary for their expression.

NMDA receptors are heterodimeric complexes assembled from NR1 and NR2 subunits. The NR1 subunit contains the glycine-binding site where as the NR2 subunit contains the glutamate (and therefore the AP5) binding site (Cull-Candy, Brickley, & Farrant, 2001). They are expressed as four different isoforms: NR2A-NR2D (Cull-Candy et al., 2001). In the amygdala of adult rat, NR2A are abundant in basolateral nuclei, whereas NR2B are more abundant in the central nucleus (Lopez de Armentia & Sah, 2003). The NR2B subunit of the NMDA receptor in the basolateral amygdala appears critical for mediating LTP and learning (Sotres-Bayon, Bush, & LeDoux, 2007; Day, Cooper, Markham, & Huhman, 2011). Although AP5 has been widely used as competitive NMDA antagonist, it does not distinguish between NR2A and NR2B subunits. Further experiments using a more NR2B-selective antagonist, such as ifenprodil, should address this issue.

NMDA-D1 receptor signaling interaction mediating flavor-nutrient preference learning

Our findings that NMDA receptor antagonism within the AMY prevented the acquisition but not expression of flavor-nutrient preference learning mirror those we recently reported with DA D1-like receptor antagonism in this forebrain area (Touzani et al., 2009), suggesting that signaling at both of these receptors within the AMY interact to promote this form of incentive learning. One way to demonstrate such an interaction is to block simultaneously DA transmission in one AMY and glutamate transmission in the contralateral AMY during training. The results of Experiment 3 demonstrate that this procedure was as effective in preventing the establishment of flavor preferences, as was bilateral NMDA or DA D1-like receptor antagonism alone. That is, administration of AP5 in one side of AMY and SCH23390 in the other side of AMY (Drug/Drug group) totally blocked the acquisition of glucose-CFP, where as unilateral administration of either drug

alone in the AMY (Drug/Vehicle group) had no effect. This is of a special interest because unilateral antagonism of AMY NMDA receptors with AP5 was sufficient to impair the acquisition of appetitive instrumental learning (Bladwin et al., 2000) and suggests that fundamental differences between flavor-nutrient learning and other forms of associative learning exist. To the best of our knowledge, these results are the first to demonstrate that co-activation of NMDA and DA D1-like receptors within AMY mediates incentive learning. The present study was not designed to elucidate the cellular and molecular mechanism underlying this interaction but several lines of evidence indicate that DA signaling at D1-like receptors within other brain areas facilitates firing of neurons elicited by glutamatergic inputs. Indeed, LTP induced in the nucleus accumbens, medial prefrontal cortex or hippocampus neurons by repeated tetanizations to slice preparations is modulated by DA D1-like receptors (Bissiere, Humeau, & Luthi, 2003; Otani, Daniel, Roisin, & Crepel, 2003; Huang, Simpson, Kellendonk, & Kandel, 2004; Schotanus & Chergui, 2008). LTP is widely considered one of the cellular mechanisms underlying learning and the mediation of learning by DA-glutamate interaction in these forebrain areas has been reported as well (Burns, Everitt, Kelley, & Robbins, 1994; Smith-Roe & Kelley, 2000; Baldwin, Sadeghian, & Kelley, 2002; Chen & Yang, 2002; Nai, Li, Wang, Liu, Lee, Frankland, & Liu, 2010). These findings indicate that learning is mediated by universal cellular and molecular mechanisms underlying DA-glutamate interaction (see (Beninger & Gerdjikov, 2004) and the results of the present study suggest that flavor-nutrient learning as well depends on these mechanisms. In adult rats, AMY neurons express both NMDA and DA D1-like receptors (Pickel et al., 2006), and DA D1-like receptors modulate NMDA currents by physical protein-protein interactions (Pickel et al., 2006; Nai et al., 2010). Thus in our learning paradigm, it is possible that DA released in the AMY and acting on DA D1-like receptors promotes flavor-nutrient preference learning by altering the effectiveness of activated glutamatergic synapses in this structure by the flavor cues. Further experiments are warranted to elucidate the cellular and molecular mechanisms underlying flavor-nutrient learning.

Obesity is recognized as a major world-wide public health problem. The etiology of obesity is complex but there is general agreement that excessive intake of palatable sugar- and/or fat-rich foods is a contributing factor. These foods are attractive in part due to unlearned taste preferences (e.g., sweet) but also in part to flavor-nutrient learning. Our recent findings indicate that sugar- and fat-CFP depend on the integrity of the AMY and that sugar conditioning also involves DA D1-like signaling in the AMY (Touzani & Sclafani, 2005; Touzani et al., 2009). The present findings further revealed that post-oral sugar conditioning depends on interactions between signaling at DA D1-like receptors and glutamate NMDA receptors in the AMY. A better understanding of the basic cellular and molecular mechanisms involved in appetite and learned food preferences may provide insights into the clinical treatment of some cases of overeating and obesity.

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References

- Andrzejewski ME, Sadeghian K, Kelley AE. Central amygdalar and dorsal striatal NMDA receptor involvement in instrumental learning and spontaneous behavior. *Behavioral Neuroscience*. 2004; 118:715–729. [PubMed: 15301599]
- Andrzejewski ME, Spencer RC, Kelley AE. Instrumental learning, but not performance, requires dopamine D1-receptor activation in the amygdala. *Neuroscience*. 2005; 135:335–345. [PubMed: 16111818]

- Asan E. Ultrastructural features of tyrosine hydroxylase-immunoreactive afferents and their targets in the rat amygdala. *Cell and Tissue Research*. 1997; 288:449–469. [PubMed: 9134859]
- Baldwin AE, Holahan MR, Sadeghian K, Kelley AE. N-methyl-D-aspartate receptor-dependent plasticity within a distributed corticostriatal network mediates appetitive instrumental learning. *Behavioral Neuroscience*. 2000; 114:84–98. [PubMed: 10718264]
- Baldwin AE, Sadeghian K, Kelley AE. Appetitive instrumental learning requires coincident activation of NMDA and dopamine D1 receptors within the medial prefrontal cortex. *Journal of Neuroscience*. 2002; 22:1063–1071. [PubMed: 11826135]
- Baxter MG, Murray EA. The amygdala and reward. *Nat Rev Neurosci*. 2002; 3:563–573. [PubMed: 12094212]
- Beninger RJ. Role of D1 and D2 receptors in learning. In: Waddington, J., editor. *D1, D2 Dopamine receptor interactions: Neuroscience and pharmacology*. London: Academic Press; 1993. p. 115–157.
- Beninger RJ, Gerdjikov T. The role of signaling molecules in reward-related incentive learning. *Neurotox Res*. 2004; 6:91–104. [PubMed: 15184110]
- Bissiere S, Humeau W, Luthi A. Dopamine gates LTP induction in lateral amygdala by suppressing feedforward inhibition. *Nature Neuroscience*. 2003; 6:587–592.
- Burns LH, Everitt BJ, Kelley AE, Robbins TW. Glutamate-dopamine interactions in the ventral striatum: role in locomotor activity and responding with conditioned reinforcement. *Psychopharmacology*. 1994; 115:516–528. [PubMed: 7871097]
- Burns LH, Everitt BJ, Robbins TW. Intra-amygdala infusion of the N-methyl-D-aspartate receptor antagonist AP5 impairs acquisition but not performance of discriminated approach to an appetitive CS. *Behavioral and Neural Biology*. 1994; 61:242–250. [PubMed: 7915108]
- Cardinal RN, Parkinson JA, Hall J, Everitt BJ. Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. *Neuroscience and Biobehavioral Reviews*. 2002; 26:321–352. [PubMed: 12034134]
- Chen L, Yang CR. Interaction of dopamine D1 and NMDA receptors mediates acute clozapine potentiation of glutamate EPSPs in rat prefrontal cortex. *Journal of Neurophysiology*. 2002; 87:2324–2336. [PubMed: 11976371]
- Cole S, Powell DJ, Petrovich G. Differential recruitment of distinct amygdalar nuclei across appetitive associative learning. *Learn*. 2001; 20:295–299.
- Cull-Candy S, Brickley S, Farrant M. NMDA receptor subunits: diversity, development and disease. *Curr Opin Neurobiol*. 2001; 11:327–335. [PubMed: 11399431]
- Day DE, Cooper MA, Markham CM, Huhman KL. NR2B subunit of the NMDA receptor in the basolateral amygdala is necessary for the acquisition of conditioned defeat in Syrian hamsters. *Behavioural Brain Research*. 2011; 217:55–59. [PubMed: 20933543]
- Goosens KA, Maren S. NMDA receptors are essential for the acquisition, but not expression, of conditional fear and associative spike firing in the lateral amygdala. *European Journal of Neuroscience*. 2004; 20:537–548. [PubMed: 15233763]
- Harmer CJ, Phillips GD. Enhanced dopamine efflux in the amygdala by a predictive, but not a non-predictive, stimulus: facilitation by prior repeated D-amphetamine. *Neuroscience*. 1999; 90:119–130. [PubMed: 10188939]
- Hatfield T, Gallagher M. Taste-potentiated odor conditioning: impairment produced by infusion of an N-methyl-D-aspartate antagonist into basolateral amygdala. *Behavioral Neuroscience*. 1995; 109:663–668. [PubMed: 7576210]
- Heffner TG, Hartman JA, Seiden LS. Feeding increases dopamine metabolism in the rat brain. *Science*. 1980; 208:1168–1170. [PubMed: 7375926]
- Huang YY, Simpson E, Kellendonk C, Kandel ER. Genetic evidence for the bidirectional modulation of synaptic plasticity in the prefrontal cortex by D1 receptors. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101:3236–3241. [PubMed: 14981263]
- Kirk, RE. *Experimental Design: Procedures for the Behavioral Sciences*. Pacific Grove, CA: Brooks/Cole; 1995.

- Lopez de Armentia M, Sah P. Development and subunit composition of synaptic NMDA receptors in the amygdala: NR2B synapses in the adult central amygdala. *Journal of Neuroscience*. 2003; 23:6876–6883. [PubMed: 12890782]
- Maren S. Long-term potentiation in the amygdala: a mechanism for emotional learning and memory. *Trends in Neurosciences*. 1999; 22:561–567. [PubMed: 10542437]
- Myers KP, Sclafani A. Conditioned enhancement of flavor evaluation reinforced by intragastric glucose: II. Taste reactivity analysis. *Physiology and Behavior*. 2001; 74:495–505. [PubMed: 11790409]
- Nai Q, Li S, Wang SH, Liu J, Lee FJ, Frankland PW, Liu F. Uncoupling the D1-N-methyl-D-aspartate (NMDA) receptor complex promotes NMDA-dependent long-term potentiation and working memory. *Biol Psychiatry*. 2010; 67:246–254. [PubMed: 19846062]
- Otani S, Daniel H, Roisin MP, Crepel F. Dopaminergic modulation of long-term synaptic plasticity in rat prefrontal neurons. *Cereb Cortex*. 2003; 13:1251–1256. [PubMed: 14576216]
- Paxinos, G.; Watson, C. *The rat brain in stereotaxic coordinates*. San Diego, CA: Academic Press; 1998.
- Pickel VM, Colago EE, Mania I, Molosh AI, Rainnie DG. Dopamine D1 receptors co-distribute with N-methyl-D-aspartic acid type-1 subunits and modulate synaptically-evoked N-methyl-D-aspartic acid currents in rat basolateral amygdala. *FASEB Journal*. 2006; 142:671–690.
- Riedel G, Platt B, Micheau J. Glutamate receptor function in learning and memory. *Behavioural Brain Research*. 2003; 140:1–47. [PubMed: 12644276]
- Sah P, Farber ESL, Lopez de Armentia M, Power J. The Amygdaloid Complex: Anatomy and Physiology. *Physiol Rev*. 2003; 83:803–834. [PubMed: 12843409]
- Schotanus SM, Chergui K. Dopamine D1 receptors and group I metabotropic glutamate receptors contribute to the induction of long-term potentiation in the nucleus accumbens. *Neuropharmacology*. 2008; 54:837–844. [PubMed: 18272187]
- Sclafani A. How food preferences are learned - laboratory animal models. *Proceedings of the Nutrition Society*. 1995; 54:419–427. [PubMed: 8524889]
- Sclafani A, Ackroff K. Nutrient-conditioned flavor preference and incentive value measured by progressive ratio licking in rats. *Physiology and Behavior*. 2006; 88:88–94. [PubMed: 16626765]
- Sigurdsson T, Doyère V, Cain CK, LeDoux JE. Long-term potentiation in the amygdala: a cellular mechanism of fear learning and memory. *Neuropharmacology*. 2007; 52:215–227. [PubMed: 16919687]
- Smith-Roe SL, Kelley AE. Coincident activation of NMDA and dopamine D1 receptors within the nucleus accumbens core is required for appetitive instrumental learning. *Journal of Neuroscience*. 2000; 20:7737–7742. [PubMed: 11027236]
- Stotes-Bayon F, Bush DE, LeDoux JE. Acquisition of fear extinction requires activation of NR2B-containing NMDA receptors in the lateral amygdala. *Neuropsychopharmacology*. 2007; 32:1929–1940. [PubMed: 17213844]
- Touzani K, Bodnar RJ, Sclafani A. Dopamine D1-like receptor antagonism in amygdala impairs the acquisition of glucose-conditioned flavor preference in rats. *European Journal of Neuroscience*. 2009; 30:289–298. [PubMed: 19614979]
- Touzani K, Sclafani A. Conditioned flavor preference and aversion: Role of the lateral hypothalamus. *Behavioral Neuroscience*. 2001; 115:84–93. [PubMed: 11256455]
- Touzani K, Sclafani A. Critical role of amygdala in flavor but not taste preference learning in rats. *European Journal of Neuroscience*. 2005; 22:1767–1774. [PubMed: 16197517]
- Wickens, J. *A theory of the striatum*. Oxford: Pergamon Press; 1993.
- Yasoshima Y, Morimoto T, Yamamoto T. Different disruptive effects on the acquisition and expression of conditioned taste aversion by blockades of amygdalar ionotropic and metabotropic glutamatergic receptor subtypes in rats. *Brain Research*. 2000; 869:15–24. [PubMed: 10865054]

Abbreviations

AMY amygdala

AP5	2-amino-5-phosphonopentanoate
BLA	basolateral amygdale
CeA	central amygdale
CS	conditioned stimulus
DA	dopamine
IG	intragastric
LTP	long-term potentiation
US	unconditioned stimulus

Highlights

- AP5 in the AMY during training prevented the acquisition of glucose-CFP.
- AP5 in the AMY during testing did not affect the previously acquired glucose-CFP.
- Uncoupling of NMDA and dopamine D1 receptors within AMY prevented glucose-CFP.
- Co-activation of NMDA-dopamine D1 receptors is necessary for Glucose-CFP.

Figure 1.

Experiment 1. Schematic representations of cannula tip placements (black circles) in the amygdala (1A). Coronal sections were adapted from Paxinos and Watson (1998) with permission. Numbers denote distance (in mm) posterior to bregma. Representative photomicrograph of a coronal section indicating bilateral cannula tracts terminating in the amygdala is shown in 1B. Scale bar: 1 mm.

Figure 2.

Experiment 1. Intakes (+S.E.M.) of the CS+ and CS- during two-bottle choice tests; data represent the mean of two 30-min sessions. Ten minutes prior to testing, the rats were injected with 0 (vehicle), 10, 20 nmol of AP5 into the amygdala (5, 10 nmol/side). The CS+ was paired with concurrent intragastric infusions of glucose and the CS- was paired with intragastric water infusions during training. No gastric infusions were given during testing. The asterisk denotes a significant ($p < 0.05$) difference between CS+ and CS- intakes. The numbers atop the bars represent the mean of the individual rat's percent CS+ intakes.

Figure 3.

Experiment 2. Schematic representations of cannula tip placements (black circles) in the amygdala of Control and AP5 rats. Coronal sections were adapted from Paxinos and Watson (1998) with permission. Numbers denote distance (in mm) posterior to bregma.

Figure 4.

Experiment 2. Intakes (+S.E.M.) of the CS+ and CS- during two-bottle choice tests; data represent the mean of four 30-min sessions. The CS+ and CS- were paired with intragastric infusions of 8 ml glucose and water, respectively, during training. No gastric infusions were given during testing. The AP5 group was given injections of 10 nmol of AP5 into the amygdala (5 nmol/side) ten minutes prior to the daily training sessions while the Control group was given vehicle injections. No injections were given prior to the two-bottle choice tests. The asterisk denotes a significant ($p < 0.05$) difference between CS+ and CS- intakes. The numbers atop the bars represent the mean of the individual rat's percent CS+ intakes.

Figure 5.

Experiment 3. Schematic representation of cannula tip placements (black circles) in the amygdala of Drug/Vehicle and Drug/Drug rats. Coronal sections were adapted from Paxinos and Watson (1998) with permission. Numbers denote distance (in mm) posterior to bregma.

Figure 6.

Experiment 3. Intakes (+S.E.M.) of the CS+ and CS- during two-bottle choice tests; data represent the mean of four 30-min sessions. The CS+ and CS- were paired with intragastric infusions of 8 ml glucose and water, respectively, during training. No gastric infusions were given during testing. The Drug/Drug group was given injections of 6 nmol of SCH23390 into one side of the amygdala and 5 nmol of AP5 into the other side of the amygdala ten minutes prior to the daily training sessions, while the Drug/Vehicle group was given vehicle injections in one side of the amygdala and either AP5 (5 nmol) or SCH23390 (6 nmol) into the other side of the amygdala. No injections were given prior to the two-bottle choice tests. The asterisk denotes a significant ($p < 0.05$) difference between CS+ and CS- intakes. The numbers atop the bars represent the mean of the individual rat's and CS+- intakes.