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Acquisition of glucose-conditioned flavor preference requires the activation of dopamine D1-like receptors within the medial prefrontal cortex in rats

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Abstract

In this study, we investigated the role of dopamine transmission within the medial prefrontal cortex (mPFC) in flavor preference learning induced by post-oral glucose. In Experiment 1, rats were trained with a flavor (CS+) paired with intragastric (IG) infusions of 8% glucose and a different flavor (CS−) paired with IG water infusions. The CS+ preference was evaluated in two-bottle tests following bilateral injection of the dopamine D1-like receptor antagonist, SCH23390, into the mPFC at total doses of 0, 12 and 24 nmol. SCH23390 produced dose-dependent reductions in CS+ intake but did not block the CS+ preference. In Experiment 2, new rats were injected daily in the mPFC with either saline or SCH23390 (12 nmol), prior to training sessions with CS+/IG glucose and CS−/IG water. In the two-bottle choice tests, SCH rats, unlike the Control rats, failed to prefer the CS+ (50 vs. 74%). Collectively, the results show that D1-like receptor activation in the medial prefrontal cortex plays a crucial role in the acquisition of flavor preference learning induced by the post-oral reinforcing properties of glucose.

Keywords

Carbohydrate; Conditioning; Forebrain; Learning; Mesolimbic; SCH23390

There is extensive evidence that animals learn to prefer the flavor of foods and fluids that provide positive nutritional consequences. This is documented by laboratory research showing that animals acquire strong and long-lasting preferences for flavored foods and fluids that either contain a nutrient or are paired with intragastric (IG) infusions of nutrients (Capaldi, 1996; Sclafani, 1999).

Flavor preference learning is a form of classical conditioning in which a cue flavor (conditioned stimulus, CS) is associated with the oral and/or post-oral reinforcing properties

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of a nutrient (unconditioned stimulus, US). The learning process by which a preference develops for a cue flavor that is mixed with an already preferred flavor (e.g., sweet taste of sugars) is referred to as flavor-flavor conditioning, whereas the learning process by which a preference develops for a cue flavor that is paired with the post-oral positive effects of a nutrient is referred to as flavor-nutrient conditioning (Capaldi, 1996; Sclafani, 1999). The most straightforward paradigm used to study conditioned flavor preferences (CFP) is to pair one flavor (the CS+) with the nutrient US and a different flavor (the CS-) with water on alternate days and then assess preference learning by presenting the CS+ and CS- flavors in a two-bottle choice test.

Flavor-nutrient learning requires the neural integration of orosensory and viscerosensory information and the formation of long-term flavor memories. To date, the brain mechanisms underlying these processes are not fully understood. Pharmacological and microdialysis studies implicate brain dopamine (DA) signaling in flavor-nutrient conditioning. Mark, Smith, Rada & Hoebel (1994) demonstrated an increase in dopamine efflux in the nucleus accumbens (NAc) elicited by the consumption of the CS+ flavor that was paired with IG carbohydrate infusions but not by the CS- flavor paired with IG water. A subsequent study by Azzara, Bodnar, Delamater & Sclafani (2001) provided further evidence of dopamine involvement in flavor-nutrient conditioning using systemic administration of D1- and D2-like receptor antagonists. These authors demonstrated that, unlike saline-treated control rats, animals treated with a D1-like receptor antagonist (SCH23390, 200 nmol/kg) during training did not exhibit any preference for the CS+ flavor that was paired with IG sucrose infusions. In contrast, the same dose of SCH23390 did not block the expression of a previously learned CS+ preference when the drug was administered at the time of two-bottle testing. Treatment with a D2-like receptor antagonist (raclopride; 200 nmol/kg), on the other hand, did not prevent the acquisition or expression of sucrose-conditioned flavor preference. These findings indicate that flavor-nutrient learning is critically dependent upon D1-like but not D-2 like receptor transmission.

There is an extensive literature on the critical role of the mesocorticolimbic DA system in reward processes and reward-related learning (Wise, 2004; Berridge, 2007). In this system, DA neurons in the ventral tegmental area (VTA) project to cortical and limbic structures including the medial prefrontal cortex (mPFC), amygdala (AMY) and the NAc (Swanson, 1982). In recent studies, we observed that injections of the D1-like receptor antagonist, SCH23390, into either the NAc or AMY blocked the acquisition but not the expression of a flavor preference conditioned by IG glucose infusions (Touzani, Bodnar, & Sclafani, 2008; Touzani, Bodnar, & Sclafani, 2009). These findings are congruent with the earlier report that peripheral injections of SCH23390 prevents flavor-nutrient learning (Azzara, Bodnar, Delamater, & Sclafani, 2001) and suggest a critical role of D1-like receptor signaling in different components of a distributed network mediating the formation of flavor-nutrient associations.

The mPFC, referring here to the prelimbic and infralimbic subdivisions, has intimate connections with the NAc and AMY and plays a crucial role in reward-related learning (Kelley, 2004; Ishikawa, Ambroggi, Nicola, & Fields, 2008). It receives dopaminergic projection from the A10 cell group of the VTA (Lindvall, Björklund, & Divac, 2010), and contains a large number of widely distributed neurons expressing mRNAs of D1-like receptors (Gaspar, Bloch, & Le Moine, 1995). Interestingly, neurochemical studies have shown an increase of DA efflux in the mPFC induced by feeding and food-related cues in both Pavlovian and instrumental learning (Hernandez & Hoebel, 1990; Izaki, Hori, & Nomura, 1999; Bassareo, De Luca, & Di Chiara, 2002), and activation of dopamine D1-like receptors in the mPFC is required for learning a sucrose-reinforced bar pressing response (Baldwin, Sadeghian, & Kelley, 2002). This prompted us to investigate, in the present study,

the role of D1-like receptor signaling in the mPFC in flavor preference conditioning by IG glucose infusions. To this end, SCH23390 was injected into the mPFC either prior to training or testing sessions. Central D2-like receptor signaling was not studied because systemic raclopride treatment failed to alter flavor conditioning by IG sugar infusions (Azzara et al., 2001). Based on our previous findings with systemic and central injections of SCH23390 (Azzara et al., 2001; Touzani et al., 2008; Touzani et al., 2009), and on the findings that intra-mPFC administration of SCH23390 impaired learning sucrose-reinforced bar pressing (Baldwin et al., 2002), we predicted that SCH23390 injections in the mPFC would impair the acquisition of a glucose-conditioned flavor preference, but would have only a marginal effect on the expression of a previously learned flavor preference.

Fifty six adult male Sprague-Dawley rats obtained from Charles River Laboratories (Wilmington, MA) or bred in our laboratory were used. They weighed 393–490 g at the time of brain surgery. The rats were individually housed in plastic cages with stainless steel wire lids (Ancare, Bellmore, NY) 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 Brooklyn College Animal Care and Use Committee and were performed in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals. All materials, procedures and testing apparatus are described in detail elsewhere (Touzani and Sclafani, 2001; Touzani et al., 2008).

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, Plastics One Inc. Roanoke, VA) were aimed at bilateral placements in the mPFC using the following coordinates: 3.0–3.2 mm anterior to Bregma, 1.3 mm lateral to the sagittal suture with a 10 degree angle and 3.4 mm ventral from the surface of the skull. The guide cannulae were secured on the skull with stainless steel 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 neck-mount 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. One rat died following the surgery in Experiment 2.

The dopamine D1-like receptor antagonist, SCH23390 (Sigma Chemical Company, St. Louis, MO) was dissolved in sterile isotonic saline (vehicle) and administered at a volume of 0.5 µl/side. Infusions of the drug or the vehicle into the mPFC were performed bilaterally using an infusion pump and 33-gauge stainless steel internal cannulae (Plastics One, Roanoke, VA) connected to 2-µl Hamilton microsyringes by polyethylene tubing. At the moment of intracerebral injections, the rats were held gently, the styli were removed and the cannulae were inserted. The tip of the injection cannulae protruded 1.0 mm beyond that of the guide. The injections were made at the rate of 0.5 µl/min and the cannulae were left in place one more minute before their removal.

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 of these 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 ($n=12$) 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. The IG infusions were performed at a rate of 1.3 ml/min and were matched in volume to the CS solutions consumed by the rats using a microcomputer and electronic lickometers. 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), 12 and 24 nmol of SCH23390 (0, 6 and 12 nmol/0.5 μ l/side) in the mPFC, 10 min prior to the two-bottle tests with the CS+ vs. CS− solutions (eight 30 min/day sessions). Half of the rats received drug injections in an ascending order, and the other half in a descending order. 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. Thus the rats received a total of four drug injections and two saline injections during testing.

In Experiment 2, the rats ($n=44$) were divided into two groups equated for their pre-training intakes of saccharin. The Control group ($n=22$) received bilateral injections of saline while the SCH group ($n=22$) received injections of 12 nmol SCH23390 (6 nmol/0.5 μ l/side) in the mPFC 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. Concurrent IG infusions that matched oral intakes started after the rat emitted 20 licks, and continuous infusions were triggered once the rat reached 300 licks to deliver the fixed volume of 8 ml. Following each pair of training sessions with CS+ and CS− solutions, there was a 1-day break. In addition, the CS+ and CS− intakes of the Control rats were limited each day to the mean intakes of the SCH 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. At the completion of the experiment, the rats were deeply anesthetized and perfused transcardially with physiological saline followed by a 10% formalin solution. The 40- μ m brain sections were prepared with a freezing microtome, stained with thionin, and cannula tracks were identified under a light microscope and reconstructed on the appropriate frontal planes of the atlas of Paxinos & Watson (1998).

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 $[(\text{CS+ intake}/\text{total intake}) \times 100]$. The data were analyzed with ANOVA or *t*-test after an arcsine transformation as recommended by Kirk (1995).

Experiment 1

Cannula tip placements for all rats used in Experiments 1 are shown in Fig. 1. Placements were deemed appropriate for all twelve rats (Fig. 1A) and were primarily localized in the prelimbic subdivision of the mPFC between the Frontal Planes +3.7 and +3.2 mm of the Paxinos & Watson (1998) atlas. A photomicrograph of a representative bilateral microinjection site is shown in Fig. 1B.

During one-bottle training, the intakes of the CS+ and CS− failed to differ significantly (12.5 and 11.7 g/30 min, respectively). In the two-bottle preference tests (Figure 2), overall, the rats consumed more CS+ than CS− [$F(1,11) = 60.9$, $p < 0.001$] and their CS intakes decreased with increasing doses of SCH23390 [$F(2,22) = 78.4$, $p < 0.001$]. Compared to intakes at the 0 nmol dose (saline), the highest dose (24 nmol) greatly suppressed total CS intakes (from 20.8 to 7.0 g/30 min). There was a CS $\times$ Dose interaction, $F(2,22) = 30.9$, $p < 0.001$ and the simple main effects analysis revealed that the rats consumed significantly ($p < 0.05$) more CS+ than CS− at all doses and that the drug dose-dependently reduced the CS+ but not the CS− intakes. Analysis of the percent CS+ intakes revealed that overall CS preferences did not decline as dose increased, and individual tests indicated that the percent CS+ intake at the highest dose was similar to that in the saline test (87% vs. 90%). Thus, SCH23390 treatment reduced CS+ intakes but did not significantly reduce the expression of CS+ preference.

Experiment 2

Four SCH rats and one Control rat did not complete testing because they stopped consuming the CS solution during training. Cannula tips were localized in prelimbic subdivision of the mPFC in fifteen Control rats and twelve SCH rats (Fig. 3) between frontal planes +3.7 and +3.2 mm of the Paxinos & Watson (1998) atlas. The remaining six SCH rats and five Control rats had either necrosis around the cannula tips or had misplaced cannulae in the anterior portion of the medial orbitofrontal subdivision and, therefore, were not included in the analysis.

The rats treated with 12 nmol SCH23390 during one-bottle training consumed only about 5 g of the CS+ or CS− during the 30-min sessions, and thus the intakes of the Control group were limited to this amount. The results of the two-bottle preference tests are summarized in Figure 4. The SCH and Control groups did not significantly differ in their total CS intakes but there was a significant Group $\times$ CS interaction [$F(1,25) = 30.90$, $p < 0.001$]. Individual comparisons revealed that the Control group consumed more CS+ than CS− ($p < 0.001$), whereas the SCH group did not differ in its intake of the CS+ and CS− solutions. The SCH rats consumed less ($p < 0.01$) CS+ and more ($p < 0.01$) CS− than did the Control rats. Consequently, the percent CS+ intake of the Control group exceeded that of the SCH group (74% vs. 50%, $t(25) = 5.42$, $p < 0.001$). The percent CS+ intakes of the six eliminated SCH rats with misplaced injection sites did not significantly differ from the Control rats (68% vs. 74%), indicating that the effects observed in the SCH group were not mediated by structures outside the mPFC. Thus, the 12 nmol dose of SCH23390 that did not impair the expression of a previously acquired glucose-conditioned flavor preference in Experiment 1 totally blocked the acquisition of this preference when administered during training in the present experiment.

In this study, we explored the role of DA D1-like receptors within the mPFC in flavor preference learning induced by the post-oral actions of glucose. The role of D2-like receptor transmission within the mPFC was not investigated because systemic treatment with the D2-like receptor antagonist, raclopride, did not impair flavor-nutrient learning (Azzara et al., 2001). The results revealed that antagonism of the DA D1-like receptors with SCH23390 (12 nmol) during training totally blocked the acquisition of the glucose-conditioned flavor preference, whereas SCH23390 treatment during testing left the expression of a previously acquired flavor preference unaffected. These findings demonstrate that activation of DA D1-like receptors in the mPFC is crucial for flavor-nutrient preference conditioning.

In Experiment 1, the rats were trained to consume a CS+ flavor paired with IG infusions of an 8% glucose solution and a CS− flavor paired with IG water infusions. In subsequent two-bottle choice tests, the rats exhibited a strong CS+ preference following vehicle (90%) and

SCH23390 (12 nmol: 93%; 24 nmol: 87%) treatments, although the drug dose-dependently reduced CS+ intake but not the consumption of the CS- that was already low under saline treatment. This drug-induced reduction in absolute CS+ intake, namely acceptance, has been observed in our previous studies following systemic and intracerebral injections of low SCH23390 doses (Azzara et al., 2001; Touzani et al., 2008; Touzani et al., 2009), and may be due to a reduction in food motivation (Yiin, Ackroff, & Sclafani, 2005).

The 12 nmol dose of SCH23390 that failed to impair the expression of a previously learned flavor preference totally blocked the acquisition of a new flavor preference when it was administered in the mPFC throughout one-bottle training (Experiment 2). Indeed, the SCH rats consumed significantly less of the CS+ and more of the CS- than the Control rats during the two-bottle preference tests. The failure of the SCH group to develop a significant CS+ preference cannot be attributed to reduced exposure to the CS+ or US (IG glucose) given that during training, the CS intakes of the Control rats were matched to those of the SCH rats and all rats were infused with the same amount of glucose. Altogether, these findings are consistent with our earlier finding that systemic injections of SCH23390 prevents flavor-nutrient preference conditioning (Azzara et al., 2001), and show that activation of dopamine D1-like receptors within the mPFC is critical for the acquisition, but not the expression, of this type of learned flavor preference. The lack of an SCH23390 effect on the expression of a previously learned flavor preference conditioned by IG glucose infusions is not surprising given that systemic treatments with this antagonist did not prevent the expression of conditioned flavor preferences induced by IG sucrose infusions (Azzara et al., 2001). This indicates that, once learned, conditioned flavor preferences based on the post-oral reinforcing properties of sugars become largely independent of dopamine signaling in the mPFC.

In the present experiments, the CS+ and CS- flavors were sweet and were similar in their hedonic and incentive values prior to training. As a consequence of being paired with IG glucose infusions during training, the CS+ flavor gained a greater hedonic value (Myers & Sclafani, 2001) and a greater incentive salience (Sclafani & Ackroff, 2006) making it more attractive and wanted than the CS- flavor. This acquired incentive salience of the CS+ flavor as well as its perception as qualitatively different from the CS- flavor were visibly preserved following D1-like receptor antagonism within the mPFC since rats that developed a strong CS+ preference after conditioning in Experiment 1, continued to exhibit significant preference for this flavor when tested under the influence of SCH23390. In Experiment 2, however, the inability of SCH rats to show preference for the CS+ flavor indicates that D1-like receptor antagonism during training prevented the CS+ from acquiring an enhanced reward value. This may have occurred because D1-like receptor antagonism blocked the reinforcing action of IG glucose infusions, prevented the association of the flavor cue with these reinforcing actions, and/or impaired the consolidation of this association. The present study was not designed to dissociate among these processes as the antagonist was administered prior to the presentation of the CS during training. Further experiments are needed to understand the role of dopamine D1-like receptor signaling within the mPFC in each of these processes that underlie glucose-conditioned flavor preferences.

Although SCH23390 has been used as dopamine D1-like receptor antagonist, it also binds with high affinity to serotonin 5-HT_{2C} receptors at which it has agonist-like properties (Millan, Newman-Tancredi, Quentric, & Cussac, 2001). Within the mPFC, 5-HT_{2C} receptors are localized on GABAergic interneurons and their stimulation by application of ligands suppresses spontaneous firing and reduces excitability of the mPFC neurons (Carr, Cooper, Ulrich, Spruston, & Surmeier, 2002). Therefore, it is possible that the blockade of the acquisition of glucose-conditioned flavor preference by SCH23390 was mainly due to stimulation of 5-HT_{2C} receptors and not by D1-like receptor antagonism. Arguing against

this possibility, we observed that bilateral microinjection of the 5-HT_{2C} receptor agonist MK 212 (0.2 µg/brain) within the mPFC during training failed to impair the acquisition of glucose-conditioned flavor preference (data not shown). Collectively, our results indicate that activation of dopamine D1-like receptors within the mPFC plays a crucial role in the acquisition of flavor preference induced by the post-oral reinforcing properties of glucose.

The mPFC of rodents is involved in several cognitive and executive functions, including working memory, temporal sequence of behavior, attention shift, and selection of behavioral strategies (Dalley, Cardinal, & Robinson, 2004). There is now growing evidence implicating DA signaling at the level of D1-like receptors within the mPFC in several Pavlovian and instrumental learning paradigms, including trace fear conditioning (Runyan & Dash, 2004), appetitive instrumental learning (Baldwin et al., 2002; Naneix, Marchand, Di Scala, Pape & Coutureau, 2009) and reinstatement of conditioned place preference behavior (Sanchez, Bailie, Wu, Li & Sorg, 2003). The findings of the present study add to this literature by highlighting the crucial role of D1-like receptor activation in the mPFC in flavor-nutrient incentive learning. They further indicate that this DA signaling is importantly involved in the acquisition/retention, but not expression/retrieval, of conditioned flavor preferences.

We recently reported that dopamine D1-like receptor antagonism within the NAc and AMY prevented the formation of flavor-nutrient preference learning induced by IG glucose infusions, but not the expression of previously learned flavor preferences (Touzani et al., 2008; Touzani et al., 2009). The striking resemblance between these findings and the results of the present study with dopamine D1-like receptor antagonism within the mPFC suggests that dopamine transmission within different discrete components of a distributed network is involved in flavor-nutrient preference learning in a complementary way. In this network, cortical and forebrain structures such as the mPFC, AMY and NAc receive dense dopamine projections from the A10 cell group of the VTA (Swanson, 1982) and these structures are interconnected via glutamatergic fibers (Christie, Summers, Stephenson, Cook, & Beart, 1987; Brog, Salyapongse, Deutch, & Zahm, 1993). Glutamatergic inputs to the NAc arise mainly from the mPFC and AMY (McGeorge & Faull, 1989; Brog et al., 1993; Zahm, 2000), and dopamine released in the NAc facilitates firing of its neurons elicited by these glutamatergic inputs (Nicola, 2007). Similarly, the mPFC and AMY receive convergent inputs from glutamatergic and dopaminergic terminals (Swanson, 1982; Kita & Kitai, 1990), and repeated tetanizations of either the mPFC or AMY neurons induces long-term potentiation that is modulated by dopamine D1-like receptors (Bissiere, Humeau, & Luthi, 2003; Otani, Daniel, Roisin, & Crepel, 2003; Huang, Simpson, Kellendonk, & Kandel, 2004; Loretan, Bissiere, & Luthi, 2004). These anatomical and electrophysiological findings are complemented by pharmacological and molecular studies showing that coordinated activation of dopamine D1-like receptors and glutamate NMDA receptors within the corticostriatolimbic network plays a critical role in reward-related learning (Kelley & Berridge, 2002). Thus, as proposed by Beninger (1993) and Wickens (1993), it is possible that dopamine released in the mPFC, AMY and NAc by nutrients or nutrient-associated cues, and acting on D1-like receptors, promotes flavor-nutrient preference learning by strengthening the effectiveness of activated glutamatergic synapses in these structures. This DA signaling may underlie different processes that develop according to a functional hierarchy within the mesocorticostratolimbic network. Indeed, evidence indicates that AMY, mPFC, and NAc neurons responded to reward-predictive cues in several learning paradigms (Muramoto, Ono, Nishijo, & Fukuda, 1993; Schoenbaum, Chiba, & Gallagher, 1998; Jodo, Suzuki, & Kayama, 2000), that NAc neuronal responses to these predictive cues require excitatory projections from the AMY and mPFC to the NAc (Ishikawa et al., 2008), and that AMY neuronal activation evoked by predictive cues precedes that of NAc neurons (Ambroggi, Ishikawa, Fields, & Nicola, 2008). The meso-amygdala dopamine system, via the D1-like receptors, may be involved in flavor-nutrient preference learning by

strengthening the association between the predictive flavor cue (CS+) and the affective significance of the reinforcing properties of nutrients (Balleine & Killcross, 2006). The meso-prefrontal cortex dopamine system may be involved in flavor-nutrient preference learning by updating the representation of the CS+ flavor in the mPFC following its association with the reinforcing actions of IG glucose (Cohen, Braver, & Brown, 2002). The meso-accumbens dopamine system may be involved in flavor-nutrient preference learning based on associations between the CS+ and the outcome of its consumption (stimulus-outcome association) as well as on the execution of actions upon the presentation of the CS+ (stimulus-action association) (Ikemoto, 2007).

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Figure 1. Schematic representations of cannula tip placements (black circles) in the medial prefrontal cortex in Experiments 1 (1A). Coronal sections were adapted from Paxinos & Watson (1998) with permission. Numbers denote distance (in mm) anterior to bregma. Representative photomicrograph of a coronal section indicating bilateral cannula tracts terminating in the medial prefrontal cortex 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), 12 or 24 nmol of SCH23390 (6 or 12 nmol/side) into the medial prefrontal cortex. 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 representation of cannula tip placements (black circles) in the medial prefrontal cortex In Experiment 2. Coronal sections were adapted from Paxinos & Watson (1998) with permission. Numbers denote distance (in mm) anterior to bregma. Scale bar: 1 mm.

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 SCH group was given injections of 12 nmol of SCH23390 (6 nmol/side) into the medial prefrontal cortex 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.