Neurobiology of Learning and Memory 94 (2010) 214–219

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

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

Khalid Touzani [a,*], Richard J. Bodnar [b,d], Anthony Sclafani [a,c,d]

[a] Department of Psychology, Brooklyn College, City University of New York, NY, USA
[b] Department of Psychology, Queens College, City University of New York, NY, USA
[c] Cognition, Brain and Behavior, City University of New York, NY, USA
[d] Neuropsychology Doctoral Subprograms, Graduate Center, City University of New York, NY, USA

ARTICLE INFO

Article history:
Received 21 April 2010
Revised 19 May 2010
Accepted 21 May 2010
Available online 1 June 2010

Keywords:
Carbohydrate
Conditioning
Forebrain
Learning
Mesolimbic
SCH23390

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.

Published by Elsevier Inc.

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 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, and 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, and 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 finding indicate that flavor–nutrient learning is critically dependent upon D1-like but not D-2 like receptor transmission.

* Corresponding author. Address: Department of Psychology, Brooklyn College of the City University of New York, 2900 Bedford Avenue, Brooklyn, NY 11210, USA. Fax: +1 718 951 4824.
E-mail address: ktouzani@brooklyn.cuny.edu (K. Touzani).

1074-7427/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.nlm.2010.05.009

There is an extensive literature on the critical role of the mesocorticolimbic DA system in reward processes and reward-related learning (Berridge, 2007; Wise, 2004). 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, 2009). These findings are congruent with the earlier report that peripheral injections of SCH23390 prevents flavor–nutrient leaning (Azzara et al., 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 (Bassareo, De Luca, & Di Chiara, 2002; Hernandez & Hoebel, 1990; Izaki, Hori, & Nomura, 1999), 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, 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 0800 h). 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 & 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 3 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 μl/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 μl/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