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

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

[a] Department of Psychology, Brooklyn College of the City University of New York, 2900 Bedford Avenue, Brooklyn, NY 11210, USA
[b] Department of Psychology, Queens College of the City University of New York, 65-30 Kissena Boulevard, Queens, NY 11367, USA

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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 remaining 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.

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1. 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 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 (Baxter & Murray, 2002; Cardinal, Parkinson, Hall, & Everitt, 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 (Andrzejewski, Spencer, & Kelley, 2005; Harmer & Phillips, 1999; Heffner, Hartman, & Seiden, 1980). 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 (Andrzejewski, Sadeghian, & Kelley, 2004; Baldwin, Holahan, Sadeghian, & Kelley, 2000). 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

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.

* Corresponding author. Fax: +1 718 951 4824.
E-mail address: ktouzani@brooklyn.cuny.edu (K. Touzani).

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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 expression and acquisition of glucose-CFP by injecting AP5 into the AMY prior either to testing or training 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; Goosens & Maren, 2004; Yasoshima, Morimoto, & Yamamoto, 2000). 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 (Beninger, 1993; Wickens, 1993).

2. Materials and methods

2.1. 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 0800 h). 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.

2.2. 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 a few days after the surgery and one rat had one brain guide-cannula clogged.

2.3. Apparatus

As detailed in Touzani and 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.

2.4. 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.

2.5. 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 μl/min and the injection cannulae were left in place an additional minute before their removal to minimize drug efflux.

2.6. 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 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 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% glu-