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Role of NMDA, opioid and dopamine D1 and D2 receptor signaling in the acquisition of a quinine-conditioned flavor avoidance in rats

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HIGHLIGHTS

- Flavored fructose, saccharin and quinine (0.03%) elicited short-term avoidance.
- Dopamine D1 antagonism during acquisition prolonged quinine-conditioned avoidance.
- Opioid antagonism during acquisition prolonged quinine-conditioned avoidance.
- NMDA antagonism during acquisition prolonged quinine-conditioned avoidance.
- Dopamine D2 antagonism during acquisition failed to affect quinine avoidance.

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ABSTRACT

A conditioned flavor preference (CFP) can be produced by pairing a flavor (conditioned stimulus, CS+) with the sweet taste of fructose. Systemic dopamine (DA) D1, D2 and NMDA, but not opioid, receptor antagonists significantly reduce the acquisition of the fructose-CFP. A conditioned flavor avoidance (CFA) can be produced by pairing a CS + flavor with the bitter taste of quinine. To evaluate whether fructose-CFP and quinine-CFA share common neurochemical substrates, the present study determined the systemic effects of DA D1 (SCH23390: SCH), DA D2 (raclopride: RAC), NMDA (MK-801) or opioid (naltrexone: NTX) receptor antagonists on the acquisition of quinine-CFA. In Experiment 1, food-restricted male rats were trained over 8 alternating one-bottle sessions to drink an 8% fructose + 0.2% saccharin solution (FS) mixed with one flavor (CS−, e.g., grape) and a different flavor (CS+, e.g., cherry) mixed in a solution (FSQ) containing fructose + saccharin and quinine at 0.001–0.030% concentrations. In six subsequent two-bottle choice tests (1–3: two sessions each) with the CS− and CS+ flavors presented in FS solutions, only rats trained with 0.03% quinine displayed a CS+ avoidance in Test 1. In Experiment 2, rats received vehicle (Veh), SCH (200 nmol/kg), RAC (200 nmol/kg), MK-801 (100 μg/kg) or NTX (1 mg/kg) 30 min prior to the 8 one-bottle training sessions with CS−/FS and CS+/FSQ (0.03% quinine) solutions. An additional vehicle group (Veh 0.06%) was trained with a CS+/FSQ containing 0.06% quinine. In the two-bottle choice tests, the Veh and RAC groups avoided the CS+ flavor in Test 1 only, whereas the SCH, MK801, and NTX groups significantly avoided the CS+ in Tests 1–3. The Veh.06% group trained avoided the CS+ in Tests 1 and 2, but not Test 3. In Experiment 3, Veh and SCH groups were trained as in Experiment 2, but were tested with CS flavors presented in 0.2% saccharin solutions. The SCH group avoided the CS+ flavor in Tests 1–3 while the Veh group avoided the CS+ in Test 1 only. Thus whereas DA D1, DA D2 and NMDA, but not opioid receptor antagonism blocked acquisition of sweet taste-based CFP, DA D1, NMDA and opioid, but not DA D2 receptor antagonism enhanced the CFA produced by the bitter taste of quinine.

1. Introduction

Animals use flavor cues (taste, odor, texture) to guide their selection of nutritious foods and avoidance of toxic foods (or fluids) with learning shaping this selection [10,39]. Four common types of food learning have been identified: conditioned flavor preferences (CFP) induced by the orosensory (flavor–taste learning; e.g., [3,4,25,39–42,59–61]) and/or

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the post-oral (flavor–nutrient learning; e.g., [1,2,47]) reinforcing properties of foods such as sugars, and conditioned flavor avoidances (CFA) induced by either ingested toxins that induce gastrointestinal distress (flavor–toxin learning; see review: [21]) or an aversive taste (flavor–taste learning; e.g., [13,14]). This paper is using the term, avoidance rather than aversion, as we did not measure taste reactivity following CFA and to allow consistency throughout the text. The fourth and least studied type of food learning, flavor–taste avoidance learning, occurs when an arbitrary flavor (CS, conditioned stimulus) is paired with a naturally unpreferred taste (US, unconditioned stimulus, e.g., bitter quinine). Fanselow and Birk [14] originally reported that rats learned to avoid a flavor (e.g., almond) mixed into a quinine solution although their study was not a pure CFA because the animals had a second flavor (e.g., vanilla) mixed into a preferred saccharin solution. More recently, Dwyer [13] trained rats to drink a CS+ flavor (e.g., cherry) added to a quinine solution and a CS− flavor (e.g., grape) added to water in separate sessions. In a subsequent two-bottle choice test, the rats avoided the CS+ when both CS flavors were presented in plain water.

Numerous studies have investigated the neurochemical substrates of flavor–taste and flavor–nutrient CFP as well as flavor–toxin CFA using dopamine (DA), NMDA and opioid receptor antagonists. In CFP studies, systemic treatment with DA D1 and D2 receptor antagonists attenuated the acquisition and expression of a flavor–taste CFP produced by the sweet taste of sucrose or fructose [4,60,61]. In contrast, systemic DA D1 but not D2 antagonism blocked the acquisition, and to a lesser degree the expression of a flavor–nutrient CFP elicited by intragastric (IG) sucrose infusions [2]. Brain sites involved in DA modulation of flavor–taste and flavor–nutrient CFP by sugar include the nucleus accumbens (NAc: [5,47]), amygdala (AMY: [6,48]) and medial prefrontal cortex (mPFC: [32,50]). In flavor–toxin CFA studies, systemic DA D1, but not D2 antagonism disrupted the acquisition of a LiCl-induced CFA [16,17]. Central drug studies revealed that DA D1 receptor antagonists administered into either the lateral hypothalamus [11] or shell of the NAc [15] disrupted the acquisition of a LiCl-induced CFA.

In NMDA receptor signaling studies, the acquisition, but not the expression of flavor–taste mediated fructose-CFP was blocked by systemic treatment with the non-competitive NMDA antagonist, MK-801 [22]. Blockade of NMDA, AMPA and metabotropic glutamate receptors in the amygdala disrupted LiCl-induced CFA [58], and blockade of NMDA receptors in the amygdala eliminated the acquisition of flavor–nutrient-CFP [51]. In contrast to DA and glutamate involvement, systemic or central administration of the general opioid antagonist, naltrexone (NTX) had little or no effect on flavor preference conditioning by the taste or nutritive actions of sugar [1,3,7,59]. However, naloxone enhanced taste aversions elicited by LiCl [12,35,45].

Flavor–taste CFA learning has not been the subject of pharmacological analysis, and the present study addressed this gap by examining the roles of DA D1, DA D2, NMDA and opioid receptor signaling in flavor avoidance conditioned by the bitter taste of quinine. In two prior studies, CFA was produced by training thirsty rats to drink flavored water adulterated with quinine [13,24]. Here we used a different design to match that used in our flavor–taste preference conditioning studies in which hungry rats were trained with a flavored fructose + saccharin solution and a less preferred flavored saccharin solution [4]. In this case, hungry rats were trained with two differently flavored fructose + saccharin (FS) solutions with one adulterated with quinine. The first experiment examined a range of quinine concentrations to determine a concentration that conditioned a flavor avoidance comparable in magnitude to the preference obtained in earlier fructose-CFP studies [3–7,22,32]. The second experiment examined the systemic effects of DA D1 (SCH23390), D2 (raclopride), NMDA (MK-801) and opioid (naltrexone) receptor antagonists on the acquisition of the quinine-induced CFA. In these experiments, the avoidance of the quinine-paired CS + flavor was evaluated in two-bottle tests with both flavored FS solutions presented without quinine. To determine if the high palatability of the FS solutions used in the choice tests may weaken the expression of the quinine conditioned avoidance, a third experiment was conducted in which the rats were given two-bottle tests using flavored saccharin solutions without fructose.

2. Methods

2.1. Subjects

Male Sprague-Dawley rats (n = 138, 250–275 g), obtained from Charles River Laboratories (Wilmington, MA), were housed individually in wire mesh cages and maintained on a 12:12 h light/dark cycle with chow (5001, PMI Nutrition International, Brentwood, MO) and water available ad libitum for the first week. All animals were then food-restricted to 85–90% of their body weight throughout behavioral testing. Food rations were provided 1 h after the end of daily training and testing sessions. The experimental protocols in the three experiments were approved by the Queens College Institutional Animal Care and Use Committee certifying that all subjects and procedures are in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2. Test solutions and initial training

The training solutions contained 8% fructose (Sigma Chemical Co., St. Louis, MO) and 0.2% sodium saccharin (Sigma Chemical Co.) with or without quinine (0.001–0.06%: Sigma Chemical Co.), each flavored with 0.05% unsweetened grape or cherry Kool-Aid (General Foods, White Plains, NY). Fructose rather than sucrose or glucose was used because, unlike these other sugars, fructose has minimal post-oral flavor conditioning effects [40–42]. Half of the rats in each group had the cherry flavor added to the fructose + saccharin (FS) solution and the grape flavor added to the fructose + saccharin + quinine (FSQ) solution; the flavors were reversed for the remaining rats. In the two-bottle choice tests, the cherry and grape flavors were presented in either 8% fructose + 0.2% saccharin (Experiments 1 and 2) or 0.2% saccharin (Experiment 3) solutions. The flavored fructose + saccharin + quinine solution is referred to as the CS+/FSQ, and the flavored fructose + saccharin solution as the CS−/FS; and the same flavors used in the two-bottle tests are referred to as CS+ and CS−, respectively. All testing took place in the rat's home cage during the mid-light phase of the light:dark cycle. The food-restricted rats were initially trained to drink an unflavored 8% fructose and 0.2% saccharin solution from sipper tubes during five daily 1-h sessions. The sipper tube was mounted on the front of the cage held by a taut steel spring, and was positioned 3–6 cm above the cage floor.

2.3. Procedure

Rats were trained over eight one-bottle training sessions (1 h) to drink the CS−/FS solution on odd-numbered days, and the CS+/FSQ solution on even-numbered days. The eight training trials were divided into four pairs of sessions with a 1-day break between each pair. In the first three training pairs, only one bottle was presented. In the fourth pair of training sessions (days 7 and 8), a second sipper tube containing water was also presented to acclimate the rats to the presence of two sipper tubes used during the choice tests; water intake was negligible in these training trials. The left–right position of the CS and water sipper tubes was counterbalanced over the 2 days. Training intakes were limited to 16 ml/session to correspond with the training procedure used in our prior fructose-CFP studies [3–7,32]. Following training, the rats were given six two-bottle choice test sessions (1 h) with unlimited access to the CS+ and CS− solutions. The position of the two bottles were left (L)-right (R)-R-L-L-R in half of the animals, and R-L-L-R-R-L in the remaining half. Solution intakes during the training and testing were measured by weighing (0.1 g) the bottles before and after the 1 h sessions.