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Operant licking for intragastric sugar infusions: Differential reinforcing actions of glucose, sucrose and fructose in mice

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HIGHLIGHTS

- C57BL/6 mice licked a dry spout for intragastric glucose but not fructose infusions.
- They adjusted their dry licking to changes in glucose concentrations and sugar type.
- FVB mice also dry licked for glucose but not fructose infusions.
- Yet, intragastric fructose conditions flavor preferences in FVB mice.
- Operant dry licking reflects nutrient-specific post-oral reinforcement.

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ABSTRACT

Intragastric (IG) flavor conditioning studies in rodents indicate that isocaloric sugar infusions differ in their reinforcing actions, with glucose and sucrose more potent than fructose. Here we determined if the sugars also differ in their ability to maintain operant self-administration by licking an empty spout for IG infusions. Food-restricted C57BL/6J mice were trained 1 h/day to lick a food-baited spout, which triggered IG infusions of 16% sucrose. In testing, the mice licked an empty spout, which triggered IG infusions of different sugars. Mice shifted from sucrose to 16% glucose increased dry licking, whereas mice shifted to 16% fructose rapidly reduced licking to low levels. Other mice shifted from sucrose to IG water reduced licking more slowly but reached the same low levels. Thus IG fructose, like water, is not reinforcing to hungry mice. The more rapid decline in licking induced by fructose may be due to the sugar's satiating effects. Further tests revealed that the Glucose mice increased their dry licking when shifted from 16% to 8% glucose, and reduced their dry licking when shifted to 32% glucose. This may reflect caloric regulation and/or differences in satiation. The Glucose mice did not maintain caloric intake when tested with different sugars. They self-infused less sugar when shifted from 16% glucose to 16% sucrose, and even more so when shifted to 16% fructose. Reduced sucrose self-administration may occur because the fructose component of the disaccharide reduces its reinforcing potency. FVB mice also reduced operant licking when tested with 16% fructose, yet learned to prefer a flavor paired with IG fructose. These data indicate that sugars differ substantially in their ability to support IG self-administration and flavor preference learning. The same post-oral reinforcement process appears to mediate operant licking and flavor learning, although flavor learning provides a more sensitive measure of sugar reinforcement.

1. Introduction

The gastrointestinal (GI) tract of mammals has many nutrient sensors for detecting the nutritional quality of ingested food. These nutrient sensors are involved in the regulation of digestion, absorption and metabolism of food as well as in the control of feeding behavior [21]. The role of gut and post-absorptive sensors in the satiation process that terminates feeding bouts has been the subject of extensive research. However, there is also considerable evidence that post-oral nutrient sensors can have an appetite stimulating effect, a process we refer to as appetition [16].

We have investigated post-oral appetition using various flavor conditioning procedures in which animals are trained to associate a novel flavor with intragastric (IG) or intestinal infusions of nutrients. In a typical experiment, rodents lick a sipper spout containing a flavored non-nutritive solution (the CS+; e.g., grape saccharin) and a computer monitoring the licks activates a pump that delivers an IG nutrient infusion (e.g., 16% glucose). In alternative sessions, the rodents lick a different flavored solution (the CS; e.g., cherry saccharin) that delivers an IG water infusion. The animals' learned association with the CS+ flavor

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and IG nutrient is revealed in a subsequent choice test in which they display a preference for the CS+ over the CS− flavor. In some experiments we have observed stimulation of licking within minutes of the first CS+ training session, indicating rapid post-oral detection of the nutrient infusion and activation of brain systems controlling food reward. Other experimental paradigms used to study post-oral nutrient influences on food reward systems include place or spout position preference conditioning in which a distinctive place or spout location is associated with an IG nutrient infusion [3,5,9].

de Araujo and coworkers recently introduced a new method to investigate post-oral nutrient control of food reward that does not involve oral ingestion or specific flavor cues. In their IG self-administration procedure mice are trained to lick a sipper spout to receive IG nutrient infusions. This method is similar to the licking-controlled infusions of flavor conditioning, except that the spout is empty during testing so there is neither flavor nor oral fluid intake. Operant "dry licking" for nutrient self-infusion without feedback from oral ingestion makes it possible to probe the sensitivity of post-oral detection of infused materials. Initial experiments demonstrated that mice adjusted their self-infusion of fat emulsions (Intralipid) to changes in fat energy concentration (0.75 to 3 kcal/ml) such that caloric intakes during 1 h sessions remained relatively constant [4,22]. Operant dry licking for nutrient infusions is not limited to dietary fat: Ferreira et al. [4] observed that mice would dry lick for IG glucose infusions at the same caloric densities as used in the fat experiment. We also studied operant dry licking for IG fat and glucose infusions and found that deletion of intestinal GPR40 and GPR120 fatty acid sensors impaired IG fat but not glucose self-administration [19].

The present study further explored the ability of mice to self-infuse nutrients in the absence of oral ingestion. In this case we tested animals with different nutritive sugars. Our IG infusion studies revealed that C57BL/6J (B6) mice display robust CS+ flavor conditioning responses to IG sucrose, glucose and glucose polymers (maltodextrin) but not IG fructose [11,12,15,25–27]. In addition, while IG glucose infusions rapidly stimulated the intake of a CS+, IG fructose infusions were ineffective. These and other findings indicate that flavor conditioning is not determined by energy value per se but by nutrient-specific factors. If operant self-infusion by dry licking is controlled primarily by nutrient energy as previously proposed [4,22], then isocaloric fructose should be as effective as glucose in maintaining this behavior in B6 mice. However, if operant self-infusion behavior is influenced by the same nutrient-specific process that controls post-oral flavor conditioning, then glucose, but not fructose, should support operant dry licking in B6 mice. If this is the case, then mouse strain differences in IG fructose flavor conditioning should also be observed in operant dry licking for fructose infusions. Unlike B6 mice, IG fructose infusions condition flavor preferences in FVB mice, although less so than isocaloric glucose infusions [18]. FVB mice, therefore, should show operant dry licking for IG fructose as well as glucose.

Experiment 1A examined the prediction that operant licking of a dry spout for IG glucose and fructose would differ in B6 mice. Experiment 1B explored the response of glucose-trained mice to changes in glucose concentration and changes in the infused sugar (i.e., to sucrose and fructose). Experiment 2 determined if the operant licking response to fructose differed from that to water and also compared the extinction of glucose-reinforced operant licking when the sugar infusion was replaced by water infusions or no infusions. Experiments 3 and 4 compared operant licking by FVB mice for IG glucose and fructose infusions at 16% and 8% concentrations, respectively.

2. Experiment 1. Operant licking for 16% glucose or fructose in B6 mice

In a previous study of operant dry licking [4], food-restricted B6 mice were trained (1 h/day) to lick a dry sipper spout for IG glucose infusions by "baiting" the spout with a food pellet. After several training sessions, the food was removed from the sipper spout and the mice continued to lick the empty spout for the IG sugar infusions alone. During training and initial empty spout tests dry licks were reinforced with IG infusions of 67.5% glucose and they were subsequently tested with IG infusions of 37.5% and 12.5% glucose.[1] We previously reported that B6 mice acquired significant preferences for a CS+ flavor paired with IG infusions of 8%, 16% or 32% glucose infusions whereas 8%, 12% or 16% fructose infusions were ineffective in producing a preference [15,26]. In the present study, therefore, we compared the operant dry licking response to isocaloric 16% glucose and fructose infusions. All mice were first trained to lick a food-baited spout which triggered IG infusions of 16% sucrose. Sucrose was selected as the training sugar because it supports flavor preference conditioning in mice and, being a glucose + fructose disaccharide, it provides post-oral exposure to both monosaccharide sugars [12,14,20].

2.1. Materials and methods

2.1.1. Animals

Adult male C57BL/6J mice were purchased from Jackson Laboratories. They were singly housed in plastic tub cages kept in a room maintained at 22 °C with a 12:12-h light–dark cycle and ad libitum access to chow (LabDiet 5001; PMI Nutrition International, Brentwood, MO) and water. During testing, they were maintained at 90% of ad libitum body weight by feeding them fixed-size chow pellets (0.5 or 1 g, Bio-Serv, Frenchtown, NJ), which allowed for precise adjustment of daily food rations. Experimental protocols were approved by the Institutional Animal Care and Use Committee at Brooklyn College and were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

2.1.2. Surgery

Mice were fitted with IG catheters (0.84 mm OD × 0.36 mm ID, Micro-Renathane tubing, MRE-033; Braintree Scientific, Braintree, MA) while anesthetized with 2% isoflurane inhalation, as previously described [14]. About 10 days after surgery, the mice were briefly (5 min) anesthetized with isoflurane, and tubing was attached to the gastric catheter and then passed through an infusion harness with a spring tether (CIH62; Instech Laboratories, Plymouth Meeting, PA). The tubing and spring were then attached to an infusion swivel mounted on a counterbalanced lever (Instech Laboratories). The body weight of each mouse was measured before and after it was fitted with the infusion tether/swivel system; daily body weights were monitored by weighing the mouse with the attached infusion tether/swivel system. Each animal was then returned to a tub cage, and the swivel's counterbalanced lever was attached above the cage.

2.1.3. Apparatus

IG infusion sessions were conducted in plastic infusion cages (15 × 15 × 32 cm) [11]. The mice licked stainless steel sipper spouts through vertical slots (5 × 20 mm, 32 mm apart) in a stainless steel plate fixed on the wall of the cage. Licking was monitored by an electronic lickometer (ENV-250B, Med Electronics, St. Albans, VT) connected to a computer, which operated syringe pumps (A-99; Razel Scientific, Stamford, CT). The pump infused liquid at a nominal rate of 0.5 ml/min into the gastric catheters as the animals licked the sipper spout, but the animal controlled the overall infusion rate and volume by its licking pattern. The pump was activated for 3 s (delivering 0.025 ml) when a criterion lick was recorded, as described in the Procedure.

2.1.4. Solutions

The solutions were prepared using food-grade sucrose (Domino Foods, Yonkers, NY), fructose and glucose (Honeyville Food Products, Rancho Cucamonga, CA). They were prepared in deionized water on a

[1] These glucose concentrations are based on the caloric densities of 2.7, 1.5, and 0.5 kcal/ml reported in [4], assuming 4 kcal/g for glucose.