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Learned flavor preferences: the variable potency of post-oral nutrient reinforcers

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Abstract

The notion that preferences for flavors paired with various nutrients can be attributed simply to their energy content (“flavor-calorie learning”) is belied by variation in nutrient reinforcing potency. Fructose, fat and ethanol, all regarded as powerful contributors to food and fluid preferences, are less potent than glucose when their orosensory effects are bypassed. Conditioning studies in animals infused with nutrients as they consume target flavor solutions have shown that the weaker reinforcing effects of these nutrients can be enhanced by various methods that improve the opportunity for associating a flavor with post-oral effects. Until the nature of the reinforcing stimuli is understood, “flavor-nutrient learning” is a better label for these phenomena.

Keywords

flavor preference learning; reinforcement

Introduction

Reinforcement (i.e., strengthening, enhancement) of food preferences has two sources. The first is oral: the flavor (taste, odor, texture) of foods is a source of information for humans and animals finding and selecting among foods. There are unlearned preferences for certain taste qualities (e.g., sweet is preferred, bitter unpreferred) that influence their initial acceptability. Preferred flavors can enhance the evaluation of other flavors that are paired with them, in an associative process known as “flavor-flavor” learning. Oral contact with food flavors is followed by post-oral effects of the nutrients, the second source of reinforcement. Both sources contribute to flavor preference learning when their effects are integrated by feeding-related brain circuits. In the associative processes of flavor-nutrient conditioning, experiencing a food flavor (conditioned stimulus) followed by a positive internal cue (unconditioned stimulus) generated by the food results in increased acceptance of and preference for that flavor in subsequent encounters. The nature of the internal cues is not yet clear, but does not appear to reflect simple provision of energy. Our work has shown that nutrients vary in their ability to reinforce these learned flavor preferences in rodents.

In an early study from another laboratory (Mehiel & Bolles, 1988), four groups of food-restricted rats were trained with flavored saccharin and one of four flavored nutrient solutions (sucrose, Polydose, ethanol and a corn oil emulsion, prepared in isocaloric concentrations) that

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differed considerably in initial acceptability to naïve animals. Each animal was given repeated exposure to one flavor (e.g., orange) in saccharin solution and another flavor (e.g., lime) in a nutrient solution. In the choice test between the training flavors presented in water, all groups showed preferences for their nutrient-paired flavor, which was interpreted as a generalized effect of calories and led to the term "flavor-calorie" conditioning. However, the magnitudes of the preferences were not necessarily identical (no statistical tests were presented), the nutrient effects were not compared directly (each animal was only trained with one nutrient), and the flavors of the nutrient sources themselves contributed to the conditioning process.

Unconditioned responses to the flavors of foods, such as the sweet taste of sugars, or the bitter quality of ethanol, can complicate the analysis of their post-oral rewarding effects. In addition to influencing the amounts animals are willing to consume, these flavors may induce flavor-flavor learning in addition to the flavor-nutrient learning about the foods. To focus on the post-oral effects of foods, we have used an intragastric flavor-nutrient learning procedure that eliminates orosensory contact with the nutrient source (Ackroff & Sclafani, 1999). As an animal consumes an arbitrary cue flavor solution (the conditioned stimulus, CS+, e.g., grape), it is infused intragastrically with a nutrient solution (the unconditioned stimulus, US). In alternate sessions a different cue flavor solution (CS-, e.g., cherry) is paired with intragastric water infusion. Preference tests are then conducted with the CS+ vs. the CS-. This procedure has several advantages. The animal controls the infusion: licks directed at the sipper tube are detected by a computer that operates a pump to infuse a matched amount of fluid. This simulates normal ingestion and prevents excessive infusion; it also halves the effective nutrient concentration in the stomach. The accompanying flavor permits cephalic responses that should normalize gut handling of the nutrient. We have used two basic procedures with this automated infusion system. For long sessions, the animal is housed in the infusion apparatus, usually with ad libitum access to chow. In long sessions the animal drinks the cue flavor solutions in multiple bouts and thus gets many paired exposures to the infusates. For short sessions, the animal is housed elsewhere and placed in the cage for daily short sessions, which is advantageous for studying short-duration effects (e.g., preloads, drugs). For short sessions the rats are usually maintained on restricted rations to motivate session intake, though we have shown that this is not necessary for flavor preference learning (Yiin, Ackroff, & Sclafani, 2005a, 2005b).

Effects of different nutrients

Of those tested thus far, the optimal nutrient for post-oral flavor preference conditioning is glucose, as well as rapidly digested glucose polymers. Polycose, a soluble maltodextrin, generates nearly exclusive preferences for the CS+ over the CS- in both long and short session procedures (Pérez, Fanizza, & Sclafani, 1999; Sclafani & Nissenbaum, 1988). In the long sessions, the flavors were unsweetened noncaloric drink mixes (Kool-Aid); these are sour and are not preferred by naïve rats relative to water. The preference for the CS+ flavor does not appear to reflect avoidance of the CS-: in tests of CS vs plain water a Kool-Aid CS- is preferred (Sclafani & Nissenbaum, 1988). In the short sessions, the CS flavors were both sweetened with saccharin to motivate intakes, and learning occurred despite the equally attractive quality of the CS+ and CS- flavors. These studies and many others used a 16% carbohydrate concentration; for Polycose, a flavor paired with 16% infusion was preferred to both 8%- and 32%-paired flavors (Lucas, Azzara, & Sclafani, 1998). Fructose, fat and ethanol are all weaker reinforcers for flavor preference learning, in two ways: the training conditions must be enhanced to obtain preferences for their paired flavors, and animals trained with two isocaloric nutrients often prefer one nutrient-paired flavor over the other.

Fructose

Because we had shown that rats drinking Polycose (a glucose polymer) and sucrose (glucose-fructose disaccharide) solutions shifted from sucrose to Polycose preference (Ackroff &

Sclafani, 1991), we wanted to evaluate fructose as a potentially weaker post-oral reinforcer. Maltose is a glucose-glucose disaccharide, suited for comparison to sucrose. We trained rats with two CS+ flavors, with 16% sucrose and maltose infusions, as well as a CS- paired with water. Both sugars conditioned strong preferences for CS+ over CS-, but the CS+maltose was strongly preferred to the CS+sucrose (Azzara & Sclafani, 1998).

Direct comparison of glucose and fructose post-oral reinforcement revealed large differences. Rats trained in the long-session procedure with unsweetened CS flavors learned strong preferences for a CS+ paired with 16% glucose infusion, but rats trained with 16% fructose did not learn after repeated training and testing. Rats trained in the short-session procedure (2 h/day) and saccharin-sweetened flavors learned strong preferences for the CS+ when trained with glucose, but rats trained with fructose acquired only a weak CS+ preference (Sclafani, Cardieri, Tucker, Blusk, & Ackroff, 1993). Another study attempted to maximize exposure to the post-oral effects of the sugars by providing chow for 2 h/day, no food or fluid for 2 h, and then 20 h of access to an unsweetened CS and paired infusion of 16% sugar solutions. Rats trained with both sugars acquired equally strong preferences for each CS+ vs. CS- (89–93%) but strongly preferred CS+glucose (86%) over CS+fructose (Ackroff, Touzani, Peets, & Sclafani, 2001). Thus far we had sweet taste and long sessions with food restriction as candidate requirements for fructose reinforcement. Using sweetened flavors in long sessions with ad libitum chow, we obtained a 71% preference for a sweet CS+ paired with 16% fructose over the CS-, suggesting that the food restriction in other studies contributed to the magnitude of the preference but was not essential to acquisition of the association (Ackroff & Sclafani, 2004). Session length is crucial, however; rats trained in 30-min sessions with a sweetened CS + paired with fructose are indifferent in preference tests vs. CS- (e.g., Sclafani, Fanizza, & Azzara, 1999).

Fat

Fat is another nutrient that has weaker post-oral reinforcing effects than glucose. Rats did not learn to prefer an unsweetened CS+ paired with oil emulsion infusion even when the emulsion concentration was increased from 7.1% (isocaloric with the standard 16% carbohydrate infusions) to 14.5 and 29% (Lucas & Sclafani, 1989). However they did learn when the flavors were saccharin-sweetened, leading us to use sweetened flavors thereafter with oil. (Enhancement by sweet taste is a recurring theme, and possible reasons for its effect are discussed later.) In direct comparisons to isocaloric carbohydrate infusions, using the technique of training with two CS+ flavors, we found equal preferences for each CS+ over CS-, but clear preferences for a CS+sucrose (Ackroff & Sclafani, 2007) or CS+maltodextrin (Lucas & Sclafani, 1999) over the CS+oil. Thus glucose-containing infusions appeared superior to oil as a flavor reinforcer.

A different picture emerged when we examined isocaloric mixed-nutrient diets high in carbohydrate (HC) or fat (HF). These were prepared from evaporated milk, maltodextrin, corn oil emulsion and water, and were isocaloric at 2.1 kcal/ml (Warwick & Weingarten, 1995). Once again, both infusates conferred strong preferences for their CS+ flavors over the CS-, but the animals preferred the CS+HF to the CS+HC (Lucas, Ackroff, & Sclafani, 1998). Noting the lower intakes of CS+HC than CS+HF during training, we next tested whether a satiating effect of the HC diet was counteracting the flavor preference. New animals were trained with the same HF diet and a diluted HC diet (1.4 kcal/ml). This equated intakes and eliminated the preference, consistent with the satiation hypothesis (Lucas, Ackroff et al., 1998). Comparing this outcome to the pure-nutrient infusions, which were only 0.64 kcal/g, we trained new animals with very diluted forms of both diets (0.5 kcal/ml), and they preferred the CS+HC over the CS+HF, a result much like that of the pure-nutrient studies (Ackroff & Sclafani, 2006).

Ethanol

Ethanol has a reputation as a reinforcer based primarily on its pharmacological effects, but its significant energy content prompted us to treat it like other nutrients. It differs from them in having a relatively unappealing orosensory effect for rats, so that bypassing its poor palatability might allow ethanol reinforcing effects to emerge. An early study (Sherman, Hickis, Rice, Rusiniak, & Garcia, 1983) using prolonged training and testing in short sessions showed that rats gradually acquired a preference for a CS+ flavor paired with a 0.5 g/kg dose of ethanol intubated to the stomach. Other rats learned a weaker preference for 1 g/kg and a clear aversion at 2 g/kg. When we used our standard flavors (0.05% Kool-aid in 0.2% saccharin) in short sessions, rats did not acquire a preference (Ackroff & Sclafani, 2003). We replicated the Sherman result using that study's flavors (0.1% HCl and 3% NaCl) and 0.5 g/kg dose when we used their sweetener (5% sucrose) but not with saccharin sweetening. More intense versions of our flavors (0.25% Kool-aid in saccharin) supported preference learning; together these data suggest that intense flavors are required for effective ethanol-based learning in short sessions.

In some of our long-session studies of ethanol post-oral reinforcement (Ackroff & Sclafani, 2001, 2002), we mimicked the sweet-fading procedure commonly used to get rats to drink ethanol (e.g., Samson, Sharpe, & Denning, 1999). However, in our case the rats were drinking saccharin-sweetened Kool-Aid and receiving paired infusions of 5% ethanol and water; the concentration of saccharin in the CS flavors was gradually reduced from 0.2% to 0%. A control group was trained with unsweetened Kool-Aids for the same 20-day period, which resulted in lower training intakes. In the preference test, conducted with unsweetened flavors for both groups, the sweet-trained group's preference was stronger (75% vs. 62% in controls). To rule out the differential training dose as a factor, new rats were trained with either a sweet CS+ (not faded) paired with 5% ethanol infusion or an unsweetened CS+ paired with 10% ethanol. This equated the average training doses (3.9 and 3.6 g/kg/day) but had opposite effects on the resulting preferences. The Sweet5 animals had a strong preference for the CS+ (89%) and the Plain10 rats avoided the CS+ (31%). As with other nutrients, ethanol-based learning was facilitated by sweetening the CS flavors.

We used training with two CS+ flavors to obtain direct comparisons of the post-oral reinforcing effects of 5% ethanol and those of isocaloric 7.18% sucrose, 7.18% fructose, and 3.26% corn oil emulsion (Ackroff, Rozental, & Sclafani, 2004). All three nutrients conferred strong (81–90%) preferences for their paired CS+ flavors over the CS–. In contrast, the CS+ethanol preferences vs. CS– were weaker (61–65%). All rats clearly preferred their CS+nutrient to the CS+ethanol. This outcome is evidence that, although it can support flavor preference learning under some circumstances, ethanol is a comparatively weak post-oral reinforcer for Sprague-Dawley rats.

Summary and conclusions

The idea that post-oral reinforcing effects of nutrients are adequately summarized as flavor-calorie learning should be rejected. Glucose and glucose polymers condition strong preferences with unpreferred flavors and in short session procedures, situations that produce poor learning with other nutrients. The three other nutrients considered here are all weaker at enhancing flavor preferences, but can be rescued by sweetening the cue flavors and by other methods that presumably increase the associability of flavor and nutrient. Fructose, despite its presence in the reinforcing disaccharide sucrose, is quite poor at reinforcing flavor preferences on its own, requiring long sessions enhanced by food restriction. Fat, in the form of corn oil emulsion, can condition flavor preferences as strong as those for glucose when measured against a CS– flavor, but in direct comparisons glucose-paired flavors are generally preferred. However, the study of mixed-nutrient diets demonstrated that at high concentrations, the satiating effects of an energy-dense high-carbohydrate diet can counteract its reinforcing effects so that the flavor

paired with an isocaloric high-fat diet is preferred. Ethanol's weak reinforcing effect relative to glucose requires intense flavors when used in short sessions. In direct comparisons, flavors associated with other nutrients are preferred to an ethanol-paired flavor.

In flavor-nutrient learning, the magnitude of the conditioned and unconditioned stimuli can be very important: a weak cue flavor or excessive nutrient infusion may impair the development of flavor preferences. For example, more concentrated carbohydrate counteracted reinforcement, and more concentrated ethanol is aversive. Sweet taste may exert its effects by making the flavor more salient and thus more memorable, which could help its association with a delayed or less intense nutrient effect. Sweet taste could also enhance cephalic responses and thus improve the handling of post-oral nutrients. Finally, sweet taste boosts the intake of the cue flavor solutions and therefore increases the exposure to their paired infusions. Note that sweet taste could also promote flavor-flavor learning, due to the association of saccharin and the cue flavors. Although this would be expected to act equally on the CS+ and CS- flavors, the enhanced response to the combined sweet+CS flavor could promote its association with nutrient effects.

Other general principles that contribute to reinforcing potency of nutrients are rapidity of post-oral effects and the training context. The post-absorptive effects of fructose and fat are delayed relative to those of glucose, which may account in part for their weaker effects. They also follow different metabolic paths, which could delay the reinforcing event; even the source of glucose reinforcement is not clear, though it may involve intestinal detection (Drucker & Scalfani, 1997; Yiin, 2006). The potency of a nutrient may depend on the overall nutrient context: training an animal with two nutrient-paired flavors can lead to different preference strengths for these flavors than when only one CS+ flavor is used (e.g., ethanol preferences are weaker when other nutrient-paired flavors are included). It is surprising that the weaker nutrients discussed here are not as reinforcing as glucose, given their presumed powerful effects on food and fluid intakes. Recent work has developed techniques for evaluating human flavor preference learning, which has shown contributions of both flavor-flavor and flavor-nutrient associations (e. g., Brunstrom, 2005; Yeomans, Leitch, Gould, & Mobini, 2008). More work in both animals and humans that focuses on the differences in reinforcing potency among nutrient sources could lead to techniques for modulating the often excessive human attraction to these foods.

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