Published in final edited form as:

Physiol Behav. 2009 June 22; 97(3-4): 381–384. doi:10.1016/j.physbeh.2009.03.007.

Flavor-Nutrient Learning is Less Rapid with Fat than with Carbohydrate in Rats

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Abstract

Flavor-nutrient learning occurs when the post-ingestive consequences of a food are associated with its flavor. As a signal of the food's energy density, flavor-nutrient associations have the potential to contribute to the regulation of meal size. While all calorie sources (fat, carbohydrate, protein, ethanol) can support flavor-nutrient learning, prior research has found that flavor-nutrient associations based on fat may require higher nutrient concentrations and more rigorous experimental protocols than are required to train carbohydrate (cho)-based associations. To further explore potential macronutrient-specific differences in flavor-nutrient learning, the present study compared the time course of acquisition of cho- and fat-based associations. Rats were trained to associate distinctive flavors with high-density (3.2kcal/mL) and low-density (0.2kcal/mL) orally-consumed solutions, either fat (corn oil emulsion) or carbohydrate (sucrose). For each nutrient, both within- and between-groups designs were used to assess (via two-bottle preference testing) whether flavor-nutrient learning had occurred after 2, 4, or 6 training trial pairs. Rats trained with carbohydrate demonstrated preferential intake of the low-density paired flavor after only 2 training pairs; in contrast, rats trained with fat required 6 training pairs. These findings demonstrate differential rapidity of acquisition flavor-nutrient associations. The longer time course of acquisition of fat-based flavor-nutrient associations may be yet another mechanism by which high-fat foods promote overeating.

Index terms

fat; carbohydrate; flavor-calorie learning; overeating; rat

Learned associations between a food's flavor and its postingestive effects are well-established phenomenon, most notably in taste aversion learning in which a food (and/or a distinctive flavor) becomes associated with unpleasant post-ingestive stimuli (e.g., nausea, vomiting, upper gastrointestinal distress) and is subsequently avoided [1,2]. In contrast, pairing a flavor with a nutrient can increase subsequent intake of that flavor relative to a flavor that is equally familiar but is not associated with nutrient [reviewed in 3,4]. Flavor-nutrient learning (also known as calorie-based learning) has been demonstrated in the laboratory by repeatedly pairing two liquids (or, less commonly, solids) differing in caloric value with distinctive cue flavors. One flavor (e.g. grape) is paired with a high-calorie nutrient or food and is thus the "high calorie flavor" (F-hi), while a second flavor (e.g. cherry) is paired with a low-calorie nutrient or food (thus, F-lo). After several (typically six) pairings of the cue flavors and caloric solutions,

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learning is assessed through a two-bottle choice test in which two bottles of calorically identical solutions (usually mid-way between the high- and low-calorie levels used in training) are given, one flavored with F-hi and one flavored with F-lo. Differential intake of F-hi and F-lo in a two-bottle test indicates a learned ingestive response to the flavors, since the solutions are nutritionally identical. Flavor-nutrient learning can be acquired through either oral [e.g. 5] or intragastric [e.g. 6] training.

Flavor-nutrient learning can exert a stimulatory effect on intake, as demonstrated in a two-bottle test when intake of a flavor previously paired with calories is greater than intake of a flavor previously paired with fewer or no calories, such as water [6-9]. All macronutrients can reinforce preferential intake, or a “flavor preference,” though the reinforcing potency differs both across macronutrients (e.g. carbohydrates tend to condition stronger preferences than fats [6,8]), as well as within a macronutrient class (e.g. safflower and corn oils tended to condition stronger preferences than beef tallow or vegetable shortening; [9]).

The flavor paired with more calories is not, however, always preferentially consumed in a two-bottle test. Greater intake of F-lo relative to F-hi has been reliably observed [10-12] and may reflect the suppression of intake of F-hi in anticipation of its potent post-ingestive effects. When considered together with other research showing preferential intake of F-hi over F-lo, one interpretation is that flavor-nutrient associations incorporate the behaviorally opposing effects of positive reinforcement (stimulatory) and anticipated satiety (inhibitory) [5,11-13]. When a flavor (F-hi) is associated with relatively few calories (but still more than F-lo), the positive reinforcing effects of the nutrient solution predominate, leading to greater intake of F-hi in a two-bottle test. However, when a flavor (F-hi) is associated with a relatively high number of calories, the satiating action of the nutrient predominates, leading to lesser intake of F-hi in the two-bottle test relative to F-lo. Other factors may contribute to lesser intake of F-hi relative to F-lo in two-bottle testing (both flavors presented in a mid-calorie solution), such as a “contrast effect” in which F-hi is devalued by its presence in the less attractive context (mid-calorie is less reinforcing than high-calorie) while F-lo's acceptance is increased by presentation in the more attractive context. Yet, greater intake of F-lo relative to F-hi is seen even when two-bottle testing is conducted with both flavors presented in the high-calorie solution [5].

Fat and carbohydrate apparently differ in terms of their relative reinforcing/satiating effects across low- and high-caloric densities. At a low caloric density (0.5 kcal/mL), intake of flavors previously paired with high-carbohydrate infusions was greater than flavors previously paired with high-fat infusions in separate two-bottle tests relative to a flavor previously paired with water infusions. However, at a high caloric density (2.1 kcal/mL), intake of flavors previously paired with high-fat infusions was greater than intake of flavors previously paired with high-carbohydrate infusions, in separate two-bottle tests relative to a flavor previously paired with water infusions [14,15]. These findings suggest that the relative reinforcing effect of fat and carbohydrate nutrient infusions depends on caloric density and/or absolute caloric value, with energy-dense, high-fat solutions being less satiating than high-carbohydrate solutions [15].

Sustained hyperphagia (consuming more calories than are expended, producing increased body fat) has been linked to consumption of a high-fat diet e.g. [16,17]. Factors contributing to high-fat diet-induced overeating include fat's higher caloric density [18,19], greater palatability e.g. [16,17,20], and less potent post-ingestive satiating effects e.g. [6,21-23], but see [24,25]. It is possible that an additional mechanism may be attenuated flavor-nutrient learning when high-fat foods are consumed, since direct comparisons of fat- vs. carbohydrate-based learning have found flavor-nutrient learning based on fat is less readily acquired [6,26,27]. Factors which influence acquisition of fat-based flavor-nutrient associations include the length of the training and testing sessions and deprivation state of the animal [28], the type of fat used [9], and the fat content of the maintenance diet [26-29].

To investigate further potential differences between fat- and carbohydrate-based flavor-nutrient learning, the present study compared the rate of acquisition of carbohydrate- and fat-based flavor-nutrient associations by varying the number of training trials. It was hypothesized that flavor-carbohydrate associations would be learned after fewer trials than flavor-fat associations. An additional hypothesis was that carbohydrate-based associations would be stronger than fat-based associations; i.e. $F-lo > F-hi$ following fat training, but $F-lo \gg F-hi$ following carbohydrate training.

Method

Subjects

165 male Long-Evans rats from Charles River, Inc. (Wilmington, MA) were used. Rats were singly housed in the temperature- and humidity-controlled colony room with a 12:12 light:dark cycle and had *ad libitum* access to tap water and Purina rodent chow (5001) at all times. Research procedures were approved by the UMBC IACUC.

Training schedule

Rats were randomly divided into eight groups; four groups were trained with fat and four with carbohydrate. For each nutrient, three groups provided a between-groups comparison of the effect of number of training trial pairs (two, four or six), while one group provided a within-groups comparison (rats received a total of six pairs of training trials, with two-bottle testing conducted after every second pair).

Training solutions

Rats were trained with either fat (corn oil: Mazola brand) or carbohydrate (sucrose: Domino brand) emulsion/solution. Each nutrient was presented in two distinctively flavored concentrations: 0.2 and 3.2 kcal/mL, chosen based on previous research demonstrating that relatively high concentrations of fat are required to promote flavor-nutrient learning [27]. Corn oil emulsions were prepared as previously described [22] using sodium steryl lactylate (Emplex: American Ingredients Inc) emulsifier at 0.6% wt/vol, and sweetened with 0.4% sodium saccharin (Sigma). Sucrose was dissolved in tap water at 5% and 80% wt/vol; the higher concentration was viscous but did remain in solution. Flavor-concentration pairings were counter-balanced within each group. Sucrose solutions were flavored with unsweetened grape or cherry Kool-Aid powder at 0.1% wt/vol and corn oil emulsions were flavored with pure lemon and almond extracts (McCormick brand) at 0.5% vol/vol.

Training procedure

Rats were given distinctively-flavored high-caloric density (3.2 kcal/mL) and low-caloric density (0.2 kcal/mL) nutrient solutions on alternating days. The number of exposure trial pairs differed by experimental group. For the “between-groups” design (separate sets of rats for fat- and cho-based training), one group received two pairs of trials, another group received four pairs of trials, and a third group received six pairs of trials. For the two “within-groups” protocols (one set of rats trained with fat, set another trained with cho), each two pairs of training trials were followed by a two-bottle test. Each rat received 30 mL of the training solution each day for 24 hours in home cages; thus, rats had equal volume exposure to the high- and low-density solutions of each nutrient. On low-density days, the training solution provided 6 kcal, while on high-density days, it provided 96 kcal.

Two bottle preference tests

After training, a two-bottle preference test was used to evaluate the acquisition of flavor-nutrient learning, in which two bottles (100 mls each) of intermediate density (1.7 kcal/ml)

solution with both flavors were presented. *Ad libitum* intake of each flavor was measured to the nearest milliliter after 30 minutes and again after 24 hours.

Data analyses

For the between-groups data, two-bottle test intakes of F-hi and F-lo were compared using paired t-tests (critical $p=0.017$). For the within-groups data, repeated measures ANOVA tested for main effects of Flavor (F-hi, F-lo), Trials (2, 4, 6), and a Flavor $\times$ Trial interaction. Rats that did not consume all training solutions were excluded from the data analysis, as they did not have equal exposure to training solutions (19, or 11% of rats were excluded). The carbohydrate-trained between-group, four training trial pairs group was repeated as data from the first group demonstrated atypically large standard errors.

Results

Carbohydrate-based training

Between-groups design—All three groups (e.g. given 2, 4, or 6 training trials) consumed less F-hi than F-lo in the two-bottle tests (Figure 1), all $p<0.017$.

Within-group design—Consistent with the between-groups design, intake of F-hi was reliably less than F-lo in the two-bottle tests conducted after 2, 4, and 6 training trials, with a main effect of Flavor, $F(1,13)=42.3$, $p<0.001$, but no effect of the number of training trials, $F(2,12)=0.20$, ns, and no interaction (Figure 2).

Fat-based training

Between-groups design—Rats receiving only two training trials did not differentially consume F-hi and F-lo in two bottle testing, $t(13)=0.46$, ns. The group receiving four training trials had a similar outcome, $t(13)=1.74$, ns. However, the group that received six training trials did consume less F-hi than F-lo, $t(18)=-6.11$, $p<0.001$ (Figure 3).

Within-groups design—While overall intake differed across trials (main effect of Trials, $F(2,12)=16.1$, $p<0.05$), intake was not systematically related to flavor (main effect of Flavor, $F(1,13)=2.7$, ns; the interaction was also not significant) (Figure 4).

Relative strength of learned associations

Only the between-groups six trial pairs data were used, as this was the only condition in which both fat- and carbohydrate-trained rats demonstrated learning. Mean intake of F-hi expressed as a percent of total 24-hour intake was 26.5% (SD=18.3%) for the fat group and 33.8% (SD=13.7%) for the carbohydrate group. Because the data were expressed as percentages, arcsine transformation [30] was used: a two-tailed t-test found no difference between groups ($t(29)=0.99$, ns).

Discussion

Rats trained with carbohydrate as the calorie source demonstrated flavor-nutrient learning after only two training trial pairs; in contrast, rats trained identically but with fat calories required six training trial pairs before demonstrating flavor-nutrient learning. Thus, fat-based flavor-nutrient associations were less rapidly acquired than carbohydrate-based associations. These results support previous research demonstrating that flavor-nutrient associations are easily conditioned with carbohydrate calories, but flavor-nutrient associations based on fat calories are less readily acquired. However, once established, there was no difference in magnitude between conditioning based on fat or carbohydrate calories in this study.

When the post-ingestive consequences of a solution become associated with its flavor, the possibility exists to regulate intake based on the predicted caloric density of a solution. Animal studies have amply demonstrated the impact of flavor-nutrient learning on short-term (single meal) intake. However, the longer-term impact of flavor-nutrient learning, i.e. on energy intake across days or weeks, has not received as much research attention.

The question of whether flavor-nutrient learning plays a role in long-term (15 day) energy intake was tested in an animal model by *preventing* the acquisition of flavor-nutrient associations in a high-carbohydrate diet (chow). Rats were repeatedly given low-, mid-, and high-calorie chow containing distinctive flavors (orange, cherry, grape). For one group, flavor-calorie pairings were consistent, e.g. low-calorie chow was always grape, mid-calorie chow was always cherry, high-calorie chow was always orange. For another group, the flavor-calorie pairings were inconsistent across days. The latter had greater weight gain despite equivalent caloric intake between groups, indicating that inconsistent flavor-calorie relationships promoted more efficient metabolic processing [31]. A follow-up study using low-, mid-, and high-density gelatins as the food source also found greater weight gain, and greater caloric intake, in the group given inconsistent flavor-calorie pairings [32].

Flavor-nutrient learning required more training trials with fat than with carbohydrate in the present study; this could be attributable to weaker postingestive effects of fat, and/or to nutrient differences in taste saliency. Prior research [33] found that sweetening fat emulsions with saccharin facilitated flavor-nutrient learning (relative to unsweetened emulsions), possibly by increasing the rate of ingestion and thus the bolus of postingestive nutrient. Fat emulsions in the present study were saccharin-sweetened for this reason; however, the carbohydrate solutions (sucrose) may have nonetheless provided more potent oral stimulation and thus a stronger US to bridge the association between the flavor and postingestive consequences. Although the present methodology could not identify the mechanism(s) underlying the longer time course of acquisition of fat-based flavor-nutrient associations, this may be another contributing factor to the hyperphagia and weight gain that is often associated with high-fat diets.

Acknowledgments

Supported by NIDDK 55367.

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Figure 1.

All three groups (e.g. given 2, 4, or 6 training trials) consumed less F-hi than F-lo in the two-bottle tests, all $p < 0.017$.

Figure 2.

Intake of F-hi was reliably less than F-lo in the two-bottle tests conducted after 2, 4, and 6 training trials (main effect of Flavor, $F(1,13)=42.3$, $p<0.001$, no effect of the number of training trials, $F(2,12)=0.20$, ns, and no interaction.)

Figure 3.

Intake of F-lo and F-hi did not differ significantly after 2 or 4 training trials. Following 6 training trials, intake of F-hi was less than F-lo, $t(18)=-6.11$, $p<0.001$.

Figure 4.

Intake of F-lo and F-hi did not differ significantly after 2, 4, or 6 training trials (main effect of Flavor, $F(1,13)=2.7$, ns.)