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Enhanced flavor–nutrient conditioning in obese rats on a high-fat, high-carbohydrate choice diet

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HIGHLIGHTS

- Rats learn to prefer flavors paired with postingestive nutrient sensing
- Rats that became obese on a high energy diet acquired stronger flavor preferences
- Sensitivity to flavor-nutrient learning is correlated with obesity

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ABSTRACT

Through flavor–nutrient conditioning rats learn to prefer and increase their intake of flavors paired with rewarding, postingestive nutritional consequences. Since obesity is linked to altered experience of food reward and to perturbations of nutrient sensing, we investigated flavor–nutrient learning in rats made obese using a high fat/high carbohydrate (HFHC) choice model of diet-induced obesity (ad libitum lard and maltodextrin solution plus standard rodent chow). Forty rats were maintained on HFHC to induce substantial weight gain, and 20 were maintained on chow only (CON). Among HFHC rats, individual differences in propensity to weight gain were studied by comparing those with the highest proportional weight gain (obesity prone, OP) to those with the lowest (obesity resistant, OR). Sensitivity to postingestive food reward was tested in a flavor–nutrient conditioning protocol. To measure initial, within-meal stimulation of flavor acceptance by post-oral nutrient sensing, first, in sessions 1–3, baseline licking was measured while rats consumed grape- or cherry-flavored saccharin accompanied by intragastric (IG) water infusion. Then, in the next three test sessions they received the opposite flavor paired with 5 ml of IG 12% glucose. Finally, after additional sessions alternating between the two flavor-infusion contingencies, preference was measured in a two-bottle choice between the flavors without IG infusions. HFHC-OP rats showed stronger initial enhancement of intake in the first glucose infusion sessions than CON or HFHC-OR rats. OP rats also most strongly preferred the glucose-paired flavor in the two-bottle choice. These differences between OP versus OR and CON rats suggest that obesity is linked to responsiveness to postoral nutrient reward, consistent with the view that flavor–nutrient learning perpetuates overeating in obesity.

1. Introduction

Learning plays a number of crucial roles in orchestrating the motivational, behavioral, and physiological aspects of appetite and eating. Individuals learn about the times and places that food becomes available, about discrete environmental cues that predict eating opportunities, and about the safety, quality, and postingestive consequences of particular foods. Learning from these experiences serves to coordinate physiological and behavioral responses to key features of the individual's food environment.

One important function of learning involves associations between a food's sensory properties and its postingestive consequences, including aversive effects of toxins and the rewarding effects of nutrients (see, e.g., reviews [1–4]). "Flavor–nutrient conditioning" is one such Pavlovian learning process through which individuals associate initially-arbitrary (or even mildly aversive) flavors in a food with rewarding physiological effects that follow as nutrients are detected in the gut and/or metabolized postabsorptively [1,5]. Because eating necessarily involves experiencing the food's flavor and other oral/cephalic sensations prior to digestion and absorption, reliable sensory-postingestive relationships make flavor cues useful for guiding food selection and

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for eliciting preparatory responses that enable efficient metabolism of nutrient-dense meals.

Experimentally, flavor–nutrient conditioning is often studied in a paradigm that pairs oral intake of a distinctively flavored solution (CS + flavor) with simultaneous intragastric (IG) infusion of a nutrient (the US, e.g., glucose), compared to an alternative flavor (CS–) of similar initial preference paired only with IG infusion of water. The IG infusion training method allows the post-ingestive reinforcing effects of the nutrient US to be unconfounded with its taste. This experience produces robust changes in evaluation of the CS+ flavor, typically evidenced by increased preference (choice of CS+ flavor over CS–) and increased acceptance (larger meals of the CS+).

These intake-stimulating effects of gut nutrient sensing are termed "appetition" in contrast to the parallel, opposing satiation responses to ingested nutrients [6]. While it has been known for some time that appetition processes exert long-term effects on choice and meal size through learning (when a flavor paired with nutrients is encountered again subsequently) recent work reveals that appetition can act more rapidly to alter flavor evaluation within a meal. Under some circumstances, the first time that a CS + flavor is accompanied by IG nutrient infusion, licking rate is stimulated within minutes and total meal size increases [7–10]. Some evidence shows that this rapid, positive feedback effect is flavor-specific [10] and thus could be key to identifying the physiological substrates of flavor–nutrient associations.

Learned preferences and preparatory appetitive responses to flavors indicative of caloric density presumably evolved in ancestral environments characterized by food scarcity. It is easy to see then how flavor–nutrient conditioning could contribute to overconsumption in the modern environment that offers unprecedented (literally super-natural) access to high-energy foods, making it important to understand the role (s) that flavor–nutrient learning may play in obesity.

Little is known about whether the onset of diet-induced obesity is linked to differences in flavor–nutrient conditioning. Such a relationship would be found if, on the one hand, obesity resulting from a history of chronic, environmentally-induced overeating subsequently impacted the mechanisms of flavor–nutrient learning. Or alternatively, normal individual differences in sensitivity to flavor–nutrient learning could act as a predisposing risk factor in the development and onset of obesity.

Considering the first possibility, there are at least two reasons to expect an effect of obesity on flavor–nutrient learning would exist. First, obesity and excess energy intake alter gut morphology and the functioning of gut nutrient sensing pathways [11,12], which could affect the perceived magnitude or temporal onset of the post-oral reinforcing effects of ingested foods. Second, the consequences of chronic overeating include lasting changes in brain function – including in critical learning and memory areas – that further exacerbate perturbations in motivation and deficits in behavioral and physiological regulation (e.g., [13–17]). A variety of evidence links overeating and obesity to altered experience of food reward, in part through adaptations in the neural circuitry mediating responses to learned environmental cues for food reward more broadly. This appears to include disturbances in reward anticipation when food expectation is cued by the environment and in the experience of reward when food is consumed [18,19], either of which could involve flavor–nutrient associative mechanisms.

Alternatively, a link between flavor–nutrient conditioning and obesity could mean that individuals who develop more robust flavor–nutrient associations or respond to them more strongly are consequently more prone to become obese, given that the learning inherently functions to promote preference and intake of energy dense foods. An analogous effect is seen in Pavlovian conditioning of appetitive responding to external food cues, in that more robust learned food cue approach is predictive of subsequent obesity proneness on a 'junk food' diet [20]. Individual differences in eventual weight gain on various high-energy diets are predicted by the changes in food choice and meal patterns that are observed in the first few days of diet exposure [21,22] which allows for the possibility that differences in learning about postingestive nutrient reinforcement play a role.

Thus, the goal of the present study was to investigate whether a correlation exists between diet-induced obesity and flavor–nutrient conditioning in rats. Obesity was induced using a high-fat, high-carbohydrate choice diet (HFHC) in which both lard and palatable maltodextrin solution were available ad libitum in addition to chow, while control rats received chow only. This HFHC choice diet effectively models many physiological and behavioral aspects of diet-induced obesity, as rats gain weight rapidly through persistently increased intake, and develop peripheral leptin resistance, impaired glucose metabolism, and altered food motivation [23–25]. This diet is especially useful as a model of human diet-induced obesity because it combines the effects of food palatability and energy density with the stimulating effects of variety and choice.

But as a general rule, not all rats are equally prone to the hyperphagic and obesogenic effects of palatable diets. Among outbred rat strains some individuals are especially susceptible to diet induced obesity, while other individuals are more diet resistant and gain considerably less weight. In some protocols the more resistant rats gain little or no more weight on a high-energy diet than chow-fed controls (e.g., [26–29]). While there is ample evidence that highly palatable, energy-dense diets promote hyperphagia and obesity, elucidating the physiological and behavioral differences between those who are more or less obesity prone is crucial for understanding these effects. Because some effects of an obesogenic diet on reward processes vary according to obesity proneness phenotype [30], we might expect effects on flavor–nutrient conditioning to differ as well. Thus, in this experiment, we classified the rats fed the HFHC choice diet into the more obesity-prone and resistant subgroups (HFHC-OP vs. HFHC-OR) based on proportional weight gain relative to pre-experimental weight. We found the HFHC choice diet to be fairly powerful at stimulating overeating, such that even the more resistant rats were on average heavier than controls. Therefore this experiment compares control rats to two subgroups that differed in the relative degree of obesity on a high energy diet. We use the abbreviation OP and OR to reflect that these are outbred rats, not the proprietary selected DIO/DR lines, and that our diet and protocol for inducing obesity differ from those often associated with the Levin DIO/DR model.

In the present experiment, following 30 weeks of feeding on the HFHC diet or chow only, all rats had IG infusion catheters surgically installed, and the HFHC-OP, HFHC-OR, and control groups were compared in a flavor–nutrient conditioning protocol that involved a distinct flavor of saccharin (CS–) accompanied by intragastric water and a differently flavored saccharin (CS+) accompanied by IG glucose. We used a variant of the conditioning protocol that has now been extensively used by Sclafani's group [7–9,31] which is designed to detect a rapid within-session stimulation of licking the first time the CS+ flavor is paired with glucose, as well as the lasting effect of that pairing on subsequent acceptance and preference for the CS+ on subsequent encounters. Briefly, this protocol involves a series of daily sessions in which rats are first trained with CS − flavor accompanied by IG water infusion to establish a baseline meal size and licking rate. Then, three consecutive sessions of CS+ flavor paired with IG glucose demonstrate the growth in CS+ acceptance as conditioning proceeds. Finally, following additional training alternating between CS– and CS+, preference for CS+ is assessed in a two bottle choice test in the absence of IG infusions. In our protocol, the infusions paired with the CS+ and CS– flavors throughout these sessions are of fixed size and delivery rate, so that the effects of a standardized postingestive stimulus on within-session intake and later learned preference can be compared across groups, unconfounded by potential baseline differences in CS intake. The three main variables of interest were 1) the degree of immediate intake stimulation during the first session wherein the CS+ flavor was accompanied by glucose, 2) the learned increase in acceptance revealed in the second and third CS+ sessions, and 3) learned preference for the CS+ flavor over CS– in the final two-bottle choice tests. Conceivably, obesity could be linked to differences in any one of these three behavioral manifestations of flavor–nutrient conditioning.