

Short communication

Robust preference for a flavor paired with intragastric glucose acquired in a single trial

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Abstract

Rats learn to prefer flavors followed by postingestive action of nutrients. This experiment demonstrates that such preferences can be acquired with only a single, brief pairing of a novel flavor with intragastric (IG) glucose infusion. Male rats with IG catheters consumed unflavored saccharin in daily 10-min sessions, and then received in counterbalanced order on separate days a single pairing of coffee- or vinegar-flavored saccharin with IG glucose, and of the opposite flavor with IG water. Rats subsequently preferred the glucose-paired flavor in a choice test in the absence of glucose infusion. Preference was robust, persisting through repeated non-reinforced testing.

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Introduction

In the course of ordinary eating, the flavor of a food reliably precedes its postingestive effects. Because flavors may be correlated with specific nutrients or toxins, animals can learn to associate flavor cues with the aversive or rewarding postingestive consequences that follow, and subsequently use those associations in food selection and in meal size control. In one such learning process termed “flavor-nutrient conditioning,” when consumption of a novel flavor is followed by postingestive physiological actions of nutrients, preference for that flavor subsequently increases (e.g., Capaldi, 1992; Fedorchak, 1997; Mehiel, 1991; Sclafani, 1999). The novel flavor is regarded as a conditioned stimulus (CS) that becomes associated with nutrient actions detected postingestively (US). The influence of these associations on subsequent eating is one means by which experience enables animals to adjust their food selection adaptively.

In order to isolate the preference-reinforcing postingestive effects of macronutrients and to study rats’ ability to

associate them with flavor cues, the confound between a nutrient’s inherent attractive taste and its caloric density is circumvented by pairing oral consumption of an arbitrary cue flavor (referred to as CS+) with infusion of a nutrient US directly into the stomach via an intragastric (IG) catheter (Elizalde & Sclafani, 1990; Sclafani, 1999). An alternative flavor (CS−) comparable in baseline preference is paired only with IG water. The measure of conditioning is preference for CS+ over CS− in a subsequent two-bottle choice. Preferential intake of the CS+ is considered a conditioned response when it is evident in the absence of the nutritive US infusion.

Flavor-nutrient conditioning can strongly alter intake. After several training trials rats typically prefer the nutrient-paired flavor over the CS− by 70–95% in two-bottle choice tests (Sclafani, 1999). Under some circumstances flavor acceptance (absolute intake) increases also, stimulating overconsumption as CS+ “meals” become larger and more frequent, characterized by increased eating rates (Myers & Sclafani, 2001; Perez, Lucas, & Sclafani, 1998). Another indicator of robustness is resistance to both forgetting and extinction. Prior work has produced preferences that were unaffected by a 30-day retention interval (Drucker, Ackroff, & Sclafani, 1994) and persisted

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through several weeks of daily non-reinforced test sessions (Drucker, Ackroff, & Sclafani, 1994; Elizalde & Sclafani, 1990).

While it is well-documented that this conditioning can strongly alter rats' reactions to flavors, prior experiments typically have used extensive training. Usually, the CS+ flavor is paired with nutrient infusion multiple times, either in several brief daily sessions or in long-term (>20 h/day) sessions, or both. In an early study with the "electronic esophagus" technique, Elizalde and Sclafani (1990) did find strong CS+ preference in the first two-bottle choice following four training sessions (two with each CS) but those sessions were 24 h/day, allowing multiple self-initiated training exposures per session. A more recent study reported increased CS+ intake during the second CS+ flavor-nutrient pairing session (Yiin, Ackroff, & Sclafani, 2005) but again those sessions lasted 20 h/day.

To date, no studies have directly addressed the question of how little training may be needed for flavor-nutrient conditioned preferences to be acquired, or how robust such preferences might be following only minimal training. Presumably, this learning would be most adaptive for foraging animals if conditioning sufficient to alter subsequent meals could occur with the first exposure to a novel flavor, as is the case with conditioned taste aversions (i.e., Logue, 1979).

The present experiment was designed to determine if preference can be acquired with a single pairing of a flavor and IG nutrient in amounts comparable to one meal. Male rats with IG catheters were accustomed to consuming unflavored saccharin in brief (10 min) daily sessions following overnight food/water restriction. Training was a single 10-min trial in which either coffee-flavored or vinegar-flavored saccharin was accompanied by IG glucose infusion, and (on a different day but in counterbalanced order) one trial with the opposite flavor paired with IG water. Two days later, rats were given a 10-min choice between both flavors in the absence of IG glucose infusion. After a 6-day retention interval the choice test (still under non-reinforced conditions) was repeated six additional times.

Methods

Subjects

Subjects were 16 adult, male Sprague–Dawley CD rats with IG Silastic catheters (1.02 mm ID, 2.16 mm OD) surgically installed under ketamine/xylazine anesthesia (65 and 10 mg/kg). The catheter was routed from the peritoneum subcutaneously to exit between the shoulders, where it was attached to a Luer-Loc connector that remained capped when not in use. After >10 days of postoperative recovery, subjects were maintained at ~95% of their preoperative weights with ~22 g chow rations delivered each evening.

Apparatus

Training and testing were performed in cylindrical HDPE infusion cages (28 cm dia. X 35 cm tall), with two brackets on the front for 50 ml drinking bottles. When a rat was placed in the cage, its catheter was attached to infusion tubing extending to a fluid swivel (Instech) over the cage on a counterbalanced lever arm, allowing freedom of movement in the infusion cage. Delivery of IG infusions was controlled by a programmable syringe pump.

Procedure

Throughout the experiment rats' access to water in the home cages was limited to the first hour following delivery of chow rations daily. This left rats somewhat thirsty and encouraged them to consume fluids in the infusion cages the following day.

Rats were accustomed to being placed in the infusion cages, drinking unflavored 0.1% saccharin, and receiving IG infusions in 10-min one-bottle sessions occurring ~4 h before chow rations each day. The left–right position of the drinking bottle was alternated daily. For the first two sessions, rats were connected to the infusion tubing but received no IG infusion. For the next six sessions, they were infused IG with 6 ml water as they drank saccharin. For these and all subsequent sessions involving IG infusions, the infusion began in the second minute of the session, delivering 0.55 ml/min for the remainder. Consumption was measured by weighing the bottles (0.1 g).

CS+ and CS– training sessions then occurred on two consecutive days, consisting of a single 10-min session of the CS+ flavor paired with 6 ml 16% glucose infused IG, and a single session of the CS– flavor with 6 ml water infused IG. CS flavors were each 0.1% saccharin flavored with coffee (0.5% w/w instant Sanka) or vinegar (1.5% w/w Weis brand cider vinegar). Prior pilot tests with different rats found these flavors to be comparable in baseline preference relative to each other, and both moderately non-preferred to unflavored saccharin. Assignment of coffee or vinegar as the CS+ or CS– was counterbalanced. Within each of those assignments, training order (CS+ first and CS– second or vice versa) was also counterbalanced. Because during these training sessions the delivery of the IG infusion was not contingent on drinking, a rat would theoretically receive the IG infusion without consuming any of the CS flavor. However this did not occur, as all rats did consume some CS+ and some CS– during training. After conditioning there was one additional session of unflavored saccharin paired with IG water.

Rats were then tested with a 10-min two-bottle choice between CS+ and CS–. The left–right positions of the two bottles were completely counterbalanced across rats with respect to both flavor (coffee or vinegar) and assignment (CS+ or CS–). During this and subsequent two-bottle tests, rats were infused with 6 ml water IG.

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