The gastrointestinal tract “tastes” nutrients: evidence from the intestinal taste aversion paradigm

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Tracy, Andrea L., Robert J. Phillips, Michael M. Chi, Terry L. Powley, and T. L. Davidson. The gastrointestinal tract “tastes” nutrients: evidence from the intestinal taste aversion paradigm. Am J Physiol Regul Integr Comp Physiol 287: R1086–R1100, 2004. First published July 15, 2004; doi:10.1152/ajpregu.00047.2004.—To develop and use a behavioral paradigm for assessments of what nutrient properties are detected by intestinal chemoreceptors, we combined features of the “electronic esophagus” preparation (Elizalde G and Sclafani A. Physiol Behav 47: 63–77, 1990) and the conditioned taste aversion protocol (Garcia J and Koelling RA. Psychon Sci 4: 123–124, 1966). In four experiments, separate groups of food-deprived rats with gastric (experiments 1–4) or duodenal (experiment 4) catheters were infused with either carbohydrates (maltodextrin) or fats (corn oil) into their stomachs or small intestines, either while they consumed nonnutritive flavored solutions (experiments 1 and 2) or in the absence of any intake (experiments 3 and 4). For some animals, one of the macronutrient infusions was paired with lithium chloride injections shown to support conventional conditioned aversions. After training, in various oral preference test trials, animals were given opportunities to taste and consume the nonnutritive solutions that had served as oropharyngeal conditioned stimuli as well as the nutrients that had been infused intragastrically, with or without poisoning, but never sampled by mouth. As previously established, preferences for the nonnutritive flavors were enhanced by association with intragastric infusions of macronutrients, with carbohydrates producing the greater preference. On first exposure to the two macronutrients for oral consumption, animals reduced their intake of the nutrient that had been previously poisoned when it was infused into the gastrointestinal tract. These results, along with additional controls, suggest that nutrient tastes detected in the intestines can be recognized centrally based on oropharyngeal gustatory stimulation.

food preference; duodenum; gustatory; macronutrients; Pavlovian conditioning

Animals use their chemical senses to identify and select the foods they eat. The roles of the chemoreceptors of the nose and mouth in such responses have been extensively studied, but comparatively little is known about what roles the visceral afferents in the rest of the alimentary canal may play in feeding decisions. It has been established, though, that the stomach and small intestine contain cells with morphological (21) and histochemical (13) features of taste receptors. Furthermore, as recently summarized in the American Journal of Physiology-Gastrointestinal and Liver Physiology thematic review series on “nutrient tasting and signaling mechanisms in the gut,” multiple experimental approaches have demonstrated that nutrients in the gastrointestinal (GI) tract are both directly and indirectly detected (10, 16, 24). In terms of receptors in the gut specifically influencing ingestive responses, it is well established that infusions of nutrients into the GI tract while an animal is consuming either nutrients or flavored solutions alter the size of meals (e.g., 4, 22) and condition preferences for paired solutions taken orally (e.g., 19, 27).

The experimental paradigm in which ingestion of arbitrary flavors is paired with intragastric (IG) infusions of particular macronutrients or diets (the “electronic esophagus” preparation) (6) has been used effectively to analyze what postoral effects different nutrients can have on the subsequent preferences for different flavors or tastes. The electronic esophagus strategy makes it practical to vary independently the stimuli in the mouth and stimuli in the GI tract, and the paradigm also makes it possible to specify the timing of the oral and postoral stimuli so that Pavlovian conditioning can be used to analyze underlying mechanisms. To take advantage of this preparation and to extend its capabilities, we have combined the electronic esophagus preparation with the classical conditioned taste aversion paradigm (11) into a conditioned intestinal taste aversion paradigm. With our combined protocol it is possible to ask whether the postoropharyngeal GI tract independently “tastes” nutrients. In the event that it does, the intestinal taste aversion paradigm also makes it possible to analyze what is learned about nutrients and how this information is used.

The present series of four experiments demonstrates that when a nutritive taste stimulus is delivered into either the stomach or the duodenum and is followed by lithium chloride-induced illness, oral preference for that same nutrient is subsequently reduced during the first period of contact by mouth. The experiments also illustrate that this GI taste may be specific to the nutrient composition of the stimuli infused, insofar as the intestinal taste aversion technique altered preference for two different macronutrients but not two pairs of nonnutritive flavor stimuli. The findings indicated that poisoning produced a preference shift in consumption of nutrients for which rats had no apparent prior oral experience. This outcome was replicated in several experiments that were designed to render implausible different potential accounts of how this preference shift might be attributable to direct contact between the IG infusates and oropharyngeal receptors at the time of poisoning.

EXPERIMENT 1: ELECTRONIC ESOPHAGUS BASELINES

Experiment 1 was designed to 1) verify our ability to use the electronic esophagus preparation by replicating the conditioned preference findings of Lucas and Sclafani (19); 2) establish...
baseline unconditioned and conditioned preferences for two macronutrient test stimuli that would be used in conjunction with the intestinal taste aversion technique; and 3) assess the practicality of reducing the pretraining phase or adaptation typically employed in the electronic esophagus experiments.

Briefly, the flavors or conditioned stimuli (CSs) provided for oral consumption were the arbitrary nonnutritive tastants used by Lucas and Sclafani (19), and the IG-delivered infusates or unconditioned stimuli (UCSs) were either fat (corn oil) or carbohydrate (maltodextrin), also employed by Lucas and Sclafani. All animals were trained to associate one Kool-Aid flavor (CS+Fat) with an IG infusion of a corn oil emulsion and a second flavor (CS+Carb) with an equicaloric maltodextrin infusion. A third flavor (CS−) was paired with IG infusion of water. A subsequent test phase consisted of three separate two-bottle choice tests in which intakes of CS+Carb, CS+Fat, and CS− were compared.

Methods

Subjects. Subjects were twelve 90-day-old (275–300 g) naive male Sprague-Dawley albino rats (Harlan Sprague Dawley, Indianapolis, IN). They were housed individually in stainless steel cages in a colony with a 12:12-h light/dark cycle (lights on 0700). Water was available at all times. Food availability was as described below.

Surgery. Approximately 1 wk after arrival, animals were fasted overnight and surgically equipped with gastric catheters. Each animal was anesthetized (pentobarbital sodium, 60 mg/kg ip), then laparotomized, and a Silastic catheter (ID 0.025 in.; OD 0.047 in.) was introduced through a puncture wound in the greater curvature of the nonglandular stomach and advanced 1 cm into the organ. The catheter was secured in place by a stay suture attached to Marlex mesh on the serosal surface of the stomach. The entry point of the catheter on the gastric wall was closed with two concentric purse-string sutures, and a serosal tunnel on the fundus of the stomach was formed around the catheter with interrupted sutures. The catheter was then passed through the left abdominal wall and tunneled subcutaneously to a backmount (Instech Solomon, Plymouth Meeting, PA). Rats returned to presurgical body weight 7–10 days postsurgery while being maintained on ad libitum chow in their home cages. Catheters were flushed daily with 0.5–1.0 ml saline beginning 24 h after surgery.

Apparatus. Conditioning procedures were conducted in four identical cylindrical chambers (height, 16 in., diameter, 12 in.), constructed of clear Plexiglas with stainless steel grid floors. Two drinking bottles were mounted on the front of the cage, 1.5 in. from the floor and 2 in. apart, with openings 1 in. × 2 in. Spouts from the bottles protruded into the center of recessed areas 2 in. deep that the rats could access inside the cage. The animal’s catheter was passed through a stainless steel spring, which was anchored to the backmount, and attached to an overhead swivel, allowing the animal to move freely about the test chamber. The tubing was attached to a 60-ml syringe pump (Razel Scientific Instruments, Stamford, CT). The syringe pump was set at an infusion rate of 1.8 ml/min. It was activated by an animal licking at one of the drinking spouts in the cage, and the infusion stopped anytime the animal did not lick the spout for 1 s. Overall, this protocol generated a ratio of 0.58 ± 0.18 ml macronutrient infused per 1.0 ml nonnutrient flavor solution consumed. The monitoring equipment and computer software (GraphicState) were purchased from Coulbourn Instruments (Allentown, PA).

Oral and IG stimuli. Oral stimuli consisted of 0.2% sodium saccharin (Sigma, St. Louis, MO) solutions flavored with 0.05% cherry, grape, or orange unsweetened Kool-Aid (Kraft Foods, White Plains, NY). The IG carbohydrate stimulus was a 16% maltodextrin solution (Polycose, Ross Laboratories, Columbus, OH). The IG fat stimulus was an emulsion of 7.1% corn oil (Mazola, Bestfoods, Englewood Cliffs, NJ) and 0.6% emulsifier (Emplex, American Ingredients, Grandview, MO). The pH values for the two solutions were 7.31 for Polycose and 7.08 for corn oil. Both solutions had a viscosity of <100 cP (Brookfield Digital Viscometer Model DV-I+). The oral stimuli paired with fat, carbohydrate, and water infusions will be referred to as CS+Fat, CS+Carb, and CS−, respectively. Flavors paired with each IG stimulus were counterbalanced across subjects.

Pretraining procedure. The rats were randomly assigned to one of two pretraining groups (n = 6 each) before being gradually reduced to 85% of their ad libitum postsurgical body weight. All rats were maintained at this weight throughout habituation, training, and testing. Based on the habituation procedure reported by Lucas and Sclafani (19), the long habituation group (group LH) was presented with two overnight exposures to ~30 ml of unflavored saccharin solution in a bottle on the front of their home cage. This exposure was followed by six daily 30-min habituation sessions in the conditioning chambers during which the animals were allowed unlimited access to the saccharin solution, but there was no IG infusion. Three daily 30-min sessions followed in which the animals were presented again with the unflavored saccharin solution and were infused IG with plain water on the schedule described above. Rats in the short habituation group (group SH) were given an abbreviated version of this procedure. Group SH received one overnight exposure to each of the three flavored CS solutions presented in a bottle on the home cage in counterbalanced order, followed by one 30-min habituation session in the conditioning chamber during which the animals were attached to the apparatus, but not presented with any oral or IG stimuli. These sessions for group SH were conducted coincident with the final four habituation days for group LH, so that training began on the same day for all animals. The rats were run in squads of four animals.

Training procedure. The training procedure was the same for both groups. All animals were weighed and catheters were flushed with ~0.5 ml saline before being attached to the infusion apparatus. Half the animals received the CS+Carb (with IG maltodextrin) on day 1 of training, and the other half received the CS+Fat (with IG corn oil). On day 2, all animals were presented with their CS− and water infusions. On day 3, animals received the opposite of what they were given on day 1 (i.e., CS+Carb and IG Polycose if they had CS+Fat and IG corn oil on day 1 and CS+Fat and IG corn oil if they had CS+Carb and IG maltodextrin on day 1). This 3-day sequence was repeated four times for a total of twelve 30-min training sessions. The placement of the drinking spout in the left or right slot was determined randomly each day. The amount of the UCS infused was determined by recording the volume on the syringe. To reduce
the possibility of oral contact with the IG infusates, as each rat was detached from the apparatus, the catheter and area around the backmount was wiped with a paper towel and the catheter was again flushed with ~0.5 ml saline. Three hours after each daily training session, the animals were fed their daily chow ration.

Test procedure. After training, all rats were given a 30-min two-bottle test session for each of six consecutive days. Half the animals were given a choice between the CS+Fat and the CS− for the first 2 days followed by the CS+Carb and the CS− for 2 days. The remaining half of the animals were given the CS+Carb and CS− test first followed by the CS+Fat and CS− test. All rats were then given the CS+Fat and the CS+Carb for 2 days. Left-right bottle position was determined randomly on the first day and alternated daily during testing. No IG infusions were given during test sessions.

Data analysis. Data in all experiments were analyzed using repeated-measures ANOVA. Individual comparisons were made using simple main effects or Newman-Keuls tests where appropriate. Significance level was set at P < .05 for all comparisons.

Results and Discussion

Three animals failed to complete the training protocol due to backmount failures. Their data were discarded. Final group sizes were n = 4 for LH and n = 5 for SH.

Training. Intake of the CS+Fat, CS+Carb, and CS− across 12 training sessions (4 per CS) is shown in Fig. 1. The animals developed a greater acceptance of the CS+Carb than either the CS+Fat or CS− in single-bottle training, although it appears that acceptance of all CSs increased over sessions. These observations were confirmed by an ANOVA, which indicated main effects of both session [F(3,15) = 6.94, P < 0.01] and CS type [F(2,10) = 14.42, P < 0.01]. Newman-Keuls analyses confirmed that intake of CS+Carb was significantly greater than intake of CS+Fat or CS−, whereas intake of CS+Fat and CS− did not differ. There was also a main effect of habituation treatment [F(1,5) = 9.67, P < 0.05] based on animals in group SH drinking more of all CSs than group LH. However, habituation treatment did not interact significantly with sessions or CS type.

Testing. No significant effect of habituation treatment was seen in the results of the test sessions; therefore data from groups SH and LH were combined for presentation and analysis. Intake data from the three two-bottle choice tests (CS+Carb vs. CS−, CS+Fat vs. CS−, and CS+Carb vs. CS+Fat) are shown in Fig. 2. Statistical analysis of each comparison indicated that animals consumed more CS+Carb than CS− [F(1,10) = 50.13, P < 0.01], more CS+Fat than CS− [F(1,10) = 5.41, P < 0.05], and more CS+Carb than CS+Fat [F(1,10) = 82.69, P < 0.01].

These results replicate the findings obtained by Lucas and Sclafani (19), demonstrating that IG corn oil and maltodextrin can be differentially associated with oral CSs and that these CSs are selected and consumed in different amounts based on their associations with the nutrient infusions. The results also establish that the pattern of flavor preferences can be obtained during testing when the duration of the habituation procedure is reduced relative to that employed by Lucas and Sclafani (19). In addition to economy, the use of a shorter duration habituation procedure should reduce animal loss as the result of catheter failure or other problems that could occur during the course of an experiment. Accordingly, the shorter duration habituation procedure employed in experiment 1 was used in each of the subsequent experiments described here.

EXPERIMENT 2: MACRONUTRIENT INTAKE ALTERED BY INTESTINAL TASTE AVERSION

As demonstrated in experiment 1, and as previously shown by Lucas and Sclafani (19), rats exhibit changes in their intake of flavored nonnutritive stimuli when these stimuli are associated with IG infusions of carbohydrates (maltodextrin) or fats (corn oil). Experiment 1 also confirmed the observation that flavors that have been followed by IG maltodextrin are preferred relative to flavors that have been followed by infusions of similar volumes of equicaloric corn oil. To the extent that the use of the electronic esophagus procedure prevents contact between IG infusates and oropharyngeal receptors, this preference for a taste paired with carbohydrate over one paired with
fat indicates that some property of these two infusates must differentiate them postorally.

Lucas and Sclafani (19) suggested that carbohydrates may be inherently more reinforcing than fats. However, it is also possible that, in addition to any difference in reinforcement, these macronutrients also affect preference by producing distinct interoceptive stimuli that are detected in the gut. To investigate this possibility more directly, in the present experiment we differentially associated the postoral stimuli produced by IG infusion of maltodextrin and corn oil, respectively, with illness (induced by injection of LiCl).

To accomplish this, naive rats were first given flavor-nutrient training, similar to that described in experiment 1. Then the rats received additional training in which licking produced two flavor CSs, one of which was paired with IG infusion of the maltodextrin, and the other paired with IG infusion of the corn oil. After each training session with one CS and IG infusate, the rats were injected with LiCl to induce mild malaise. After training sessions with the other CS and IG infusate, the rats were given an intraperitoneal saline injection. The effects of this differential training were evaluated with three types of two-bottle preference tests as well as with a single-bottle test. The different tests were designed to determine whether this type of intestinal taste aversion procedure altered oral preference for 1) the CSs that were consumed during intestinal taste aversion training of the IG infusates; 2) the originally trained CSs that were associated with IG infusions, but were not consumed during intestinal taste aversions training; and 3) the previously unconsumed macronutrient that had been infused IG and poisoned.

Methods

Subjects. Subjects were 32 naive animals of the same description and maintained under the same conditions as experiment 1. Two procedurally identical replications of 16 animals each were run.

Surgery and apparatus. Animals underwent the same surgical procedures and were trained and tested using the apparatus described for experiment 1.

Stimuli. Oral stimuli were 0.2% sodium saccharin solutions flavored with grape or cherry (first phase) and lemon-lime or orange (second phase) Kool-Aid. IG stimuli were the same nutrient solutions described in experiment 1. LiCl (0.15 M; Sigma, St. Louis, MO) was delivered intraperitoneally at a dose of 20 ml/kg. The pairings of flavored oral stimuli with IG infusates and drug stimuli were counterbalanced across animals.

Procedure. After recovery from surgery, animals were reduced to 85% of their ad libitum postsurgical body weights. They then received one overnight exposure each to 30 ml of the grape- and cherry-flavored saccharin solutions presented in counterbalanced order in the home cage. The animals then had one 30-min habituation session in which they were placed in the conditioning chamber and attached to the apparatus, but no oral or IG stimuli were presented. After this were eight daily 30-min training sessions in which the animals were provided with either the CS+Carb and IG maltodextrin or the CS+Fat and IG corn oil. The CS-nutrient pairs were presented on alternating days, for a total of four training sessions with each nutrient. The CS-nutrient pair used on the first day was counterbalanced across animals.

Animals were then given a single 30-min test session in which they were presented with both the CS+Fat and the CS+Carb, but there were no IG infusions. After this first test session, animals were given exposure to the novel lemon-lime- and orange-flavored solutions. First, they were given a 30 ml overnight exposure to each flavor in the home cage followed by one 30-min exposure session with each flavor in the conditioning chamber (counterbalanced order, no IG infusion). Animals were then given two aversion-training sessions. In these sessions, the animals received one of the new oral stimuli (counterbalanced) to drink paired with IG maltodextrin in one session and the other flavor paired with IG corn oil in the second session (counterbalanced order). For half of the animals, after the session in which they received IG maltodextrin, they were given an intraperitoneal injection of LiCl and given an intraperitoneal injection of saline after the session in which they received IG corn oil. The other half of the animals received the reverse nutrient-drug contingency (corn oil-LiCl, maltodextrin-saline). To assess the possibility that these nutrients are detected in the gut over different time courses, half of the animals in each of these groups received injections 15 min after the end of the session, while the other half received injections 4 h after the end of the session.

One 30-min test session was then conducted in which the animals were presented with the lemon-lime and orange solutions with no IG infusions. The 3-day aversion training and two-bottle sequence was then repeated. Animals were then tested with the original CS+Carb and CS+Fat in a 30-min two-bottle test with no IG infusions. After this, two sets of single bottle tests were given: first, the CS+Carb and CS+Fat paired with the appropriate IG infusion (one session each, counterbalanced order), then the CS+Carb and CS+Fat alone with no IG infusions (one session each, counterbalanced order). Finally, all animals were presented with a two-bottle test in which they were allowed to consume orally the two infusates from bottles on the front of the home cage. Intake of the infusates was measured at 30 min and 1, 2, and 4 h.

Results and Discussion

Flavor-nutrient training. CS+Carb and CS+Fat intakes across the four flavor-nutrient training sessions are shown in Fig. 3. These data are similar to those in experiment 1, with greater intake of the CS+Carb than CS+Fat, but increasing intake of both CSs across training sessions. A repeated-measures ANOVA yielded main effects of session [F(3,90) = 53.47, P < 0.01] and CS type [F(1,30) = 61.56, P < 0.01], confirming the above observations. Poisoned nutrient (fat vs. carbohydrate) was included as a dummy variable. The lack of any effect of this variable indicates that both fat-poisoned and carbohydrate-poisoned groups demonstrated equivalent flavor-nutrient learning.

CS+Carb vs. CS+Fat pretest. Figure 4A shows the results of the CS+Carb vs. CS+Fat two-bottle test before any aversion training. This figure depicts the data based on whether the CS was paired with the nutrient that would be subsequently paired with LiCl (poisoned) or with saline (nonpoisoned) and shows that before aversion training there is no difference in intake of the CSs based on this variable. This was confirmed by an
ANOVA that yielded a main effect of CS type \(F(1,30) = 154.75, P < 0.01\), but no main effects or interactions involving poisoned nutrient type (fat vs. carbohydrate, largest \(F = 0.04\)). This indicated that the animals showed a strong preference for the CS+Carb over the CS+Fat, as in experiment 1, but that this preference was equal for the animals that would have the corn oil infusate paired with LiCl and those that would have the maltodextrin infusate paired with LiCl.

Postaversion tests. After the first LiCl training period in the first replication, there was no significant effect of poisoning on intake of the directly associated flavor (i.e., the lemon-lime or orange flavor that was consumed during the session) in the subsequent two-bottle test, indicating that the procedure was ineffective at conditioning a flavor aversion. This led to the concern that the treatment may also have been insufficient to develop an aversion to the IG nutrient. Therefore, the aversion training procedure was repeated and a second two-bottle flavor test (lemon-lime vs. orange) was conducted to assess taste aversion. As shown in Fig. 4B, there was a strong flavor aversion conditioned after two rounds of aversion training, as indicated by much greater intake of the nonpoisoned flavor. An ANOVA was run on the data from this second test and yielded a main effect of poisoning \(F(1,28) = 41.85, P < 0.01\), indicating that the LiCl was effective at conditioning a flavor aversion. There was also a reliable interaction between poisoned nutrient type and poisoned vs. nonpoisoned solution \(F(1,28) = 6.13, P < 0.05\). This interaction was due to a significant difference in intake of the nonpoisoned flavor based on its associated nutrient (specifically, if this was the carbohydrate-paired flavor, intake was greater than if this was the fat-paired flavor), which was not true for intake of the poisoned flavor (intake was the same regardless of whether fat or carbohydrate was the poisoned nutrient). This again indicated a preference for a carbohydrate-paired flavor, but not necessarily differential aversion learning, as preference for the poisoned flavor was reduced equally regardless of its paired nutrient.

Figure 4C depicts the results of the postaversion two-bottle test, in which these two solutions were again presented to the animals in the absence of any IG infusion. The results of this test were very similar to those seen in the preaversion test, with poisoning of the paired nutrient having little effect on intake of these previously associated flavors. Confirming this, an ANOVA yielded no effect of poisoning \(F(1,28) = 0.81, P > 0.05\) but did yield a main effect of CS type \(F(1,28) = 157.7, P < 0.05\), indicating that the overall preference for CS+Carb compared with CS+Fat was maintained and not affected by having had one of these nutrients poisoned. In addition, single-bottle tests in which the animals were presented with either the CS+Carb or CS+Fat in separate sessions, one with no IG infusion and one with the appropriate IG infusion, produced no differences in intake based on whether the animals had been poisoned with IG corn oil or IG maltodextrin (data not shown, largest \(F = 0.68, P > 0.05\)).

Figure 5 shows the results from the first 30 min of the two-bottle test comparing consumption of the corn oil infusate with the maltodextrin infusate when animals were allowed to consume these solutions orally for the first time. Mean intake

![Fig. 3. Mean intake of flavor CSs paired with IG infusion of 7.1% corn oil (CS+Fat) or 16% maltodextrin (CS+Carb) across four 30-min training sessions with each infusate.](image)

![Fig. 4. Mean intake of flavor CSs previously trained with the poisoned or nonpoisoned IG infusate (A and C) or paired with these IG infusates during aversion training (B) in a 30-min two-bottle choice test (conducted in extinction) either before (A) or after (B and C) aversion training of the IG infusates. *Significantly different from nonpoisoned, \(P < 0.05\).](image)
of each infusate for the poisoned and nonpoisoned treatment conditions, along with statistical analysis, is presented in the figure legend. Analysis of these data yielded a main effect of poisoning (poisoned vs. nonpoisoned solution) \(F(1,27) = 10.25, P < 0.01\), indicating that, overall, animals consumed more of the infusate paired with saline than that paired with LiCl. In addition, there was an interaction between poisoned intake and poisoned nutrient type (fat poisoned vs. carbohydrate-poisoned) \(F(1,27) = 93.65, P < 0.01\), reflecting the fact that all animals consumed more of the maltodextrin solution, but that the difference between maltodextrin and corn oil intake was smaller for animals that had IG maltodextrin poisoned. Finally, there was a significant interaction between poisoning and injection delay \(F(1,27) = 5.59, P < 0.05\), indicating that animals given IG maltodextrin-LiCl training drank less during this test if they were injected at 15 min compared with 4 h, while animals given IG corn oil paired with LiCl drank less of the infusates if they had been injected at 4 h compared with 15 min. This may reflect differences in the temporal parameters of the stimulation produced by the two IG infusates. That is, the postoral stimulus effects of the maltodextrin solution may have been more readily detected, and thus associated with the illness, at 15 min postinfusion, while the corn oil was more readily detected after 4 h. However, this interpretation is not entirely supported by these results, as there did not seem to be greater learning about the stimuli at different times (i.e., greater suppression of the poisoned solution relative to the nonpoisoned solution), only greater overall suppression of both solutions.

Intake differences between poisoned vs. nonpoisoned infusates, although comparable in direction to those obtained at 30 min, did not always achieve statistical significance at the 1-, 2-, or 4-h time points in experiment 2 or in the subsequent experiments that are reported here. Accordingly, the remaining analyses of oral preference for the infusates will be confined to the initial 30 min of testing. A number of factors may have diminished the effects of training over longer test periods. For example, it is possible that intestinal taste aversion extinguished during prolonged nonreinforced exposure to orosensory or IG stimulation or that rats experienced generalization decrement along temporal or intensity dimensions related to differences in duration of intestinal stimulation between training and testing. Furthermore, prolonged exposure during testing may result in habituation or adaptation of GI receptors, thereby altering sensitivity to nutrient stimulation.

In summary, when animals had an opportunity to sample orally and ingest the two macronutrients (one of which had been poisoned) for the first time, they showed a significant reduction in preference for the poisoned nutrient. This indicates that 1) postoral stimulation by corn oil and maltodextrin is detectable and the two nutrients can be discriminated from one another and 2) learning about these postoral consequences can be translated into effects on oral consumption, even in the absence of prior oral experience with these solutions. In contrast, the prediction that the animals would learn an aversion to stimulation produced by gastric nutrient infusions and demonstrate that aversion by avoidance of arbitrary nonnutritive CSs associated with that same nutrient stimulus was not supported by the results of this experiment. In fact, in all tests of CS intake, preference for the CS + Carb was consistently demonstrated and seemed to be unaffected by which nutrient had been paired with LiCl.

**EXPERIMENT 3: LEARNING ABOUT INTESTINAL TASTES—TWO CONTROLS**

Experiment 2 demonstrated that, at their first opportunity to taste and consume by mouth a macronutrient that had previously been infused into the GI tract and associated with malaise, animals exhibited a reduced preference for that macronutrient. At the same time, such animals did not show an effect of IG nutrient poisoning on the intake of a previously associated nonnutritive flavor CS. In the present experiment, naive subjects were used to 1) evaluate a learned safety hypothesis, in the present experiment we conducted intestinal taste aversion training first, pairing IG infusions of one of the two macronutrients with LiCl injections and the other with saline in the absence of any oral stimuli to maximize learning about the postoral nutrient stim-
ulation. The animals were then trained in the “electronic esophagus” or flavor-nutrient learning paradigm. We predicted that the postoral stimulation of the nutrient poisoned during the prior intestinal taste aversion training would be a less effective UCS for flavor preference conditioning than the nonpoisoned nutrient.

Finally, we used a summation test as a further means of assessing learning about flavor CSs that have been associated with different IG UCSs. If poisoning the IG UCS has a decremental effect on the ability of its associated flavor CS to elicit intake, then adding this CS flavor to the poisoned infusate should reduce intake of that infusate more than adding the flavor CS that was not associated with the poisoned IG UCS.

**Methods**

**Subjects.** Subjects were 24 naive animals of the same description and maintained under the same conditions as described for experiment 1.

**Surgery and apparatus.** Animals underwent the same surgical procedures and were trained and tested using the same apparatus as described for experiment 1.

**Stimuli.** Oral and IG stimuli were the same as those used in experiment 1.

**Procedure.** After recovery from surgery, animals were reduced to 85% of their ad libitum postsurgical body weights. They then received two 30-min habituation sessions in which they were placed in the conditioning chamber and attached to the apparatus, but no oral or IG stimuli were presented. Animals were then given four aversion-training sessions. In these sessions, animals were placed in the infusion cages for 20 min and infusion of either 7.1% corn oil emulsion (2 sessions) or 16% maltodextrin (2 sessions) was administered for 10 s out of every minute at a rate of 1.8 ml/min for a total infusion volume of 6 ml. The session length and infusion volumes were based on data from aversion sessions in experiment 2, in which animals determined their own infusion volumes. Half of the rats were given an intraperitoneal injection of LiCl after the sessions in which they received IG maltodextrin and were given an intraperitoneal injection of saline after the session in which they received IG corn oil. The remaining rats received the reverse nutrient-drug contingency (corn oil-LiCl, maltodextrin). After these pairings, all rats were given eight flavor-nutrient conditioning after intestinal taste aversion training with one of the two IG nutrients. Because we were interested in the effects of IG nutrient poisoning on flavor-nutrient learning, these data were presented as CS+poisoned nutrient and CS+nonpoisoned nutrient. Analysis of these data yielded no effect involving this poisoning variable [largest $F = 1.51$], indicating that flavor-nutrient learning was not affected by one of the IG nutrients being previously paired with LiCl. There was a main effect of nutrient [$F(1,20) = 20.64, P < 0.01$], due to an overall greater intake of the CS+Carb, and a main effect of session [$F(3,60) = 121.69, P < 0.01$], due to increasing intake as training progressed. These results indicate

**Results and Discussion**

**Flavor-nutrient training.** Figure 6A shows the results of four sessions of flavor-nutrient conditioning after intestinal taste aversion training with one of the two IG nutrients. Because we were interested in the effects of IG nutrient poisoning on learning these flavor associations, these data are presented as CS+poisoned nutrient and CS+nonpoisoned nutrient.
that this flavor-nutrient training was very similar to that seen in experiments 1 and 2, in which there was no prior experience with the IG nutrients. Figure 6B shows the results of the two-bottle CS+Carb vs. CS+Fat extinction test. There was no main effect or interaction involving poisoning [largest $F = 1.59$], but again these data yielded a main effect of CS type [$F(1,20) = 112.97, P < 0.01$], with CS+Carb being strongly preferred over CS+Fat, as observed previously.

Infusate oral intake test. Intake during the first 30 min that the animals were offered the two infusates (previously only administered IG) for oral consumption is presented in Fig. 7. Mean intake of each infusate for the poisoned and nonpoisoned treatment conditions, along with statistical analysis, is presented in the figure legend. As in experiment 2, oral intake of the previously poisoned nutrient was suppressed relative to the nonpoisoned nutrient. ANOVA confirmed this observation, indicating a main effect of poisoning [$F(1,22) = 16.86, P < 0.01$]. There was also a main effect of nutrient [$F(1,22) = 39.44, P < 0.01$], indicating that animals consumed more of the carbohydrate solution than the fat solution regardless of which nutrient was poisoned. Furthermore, ANOVA yielded a significant nutrient $\times$ poisoned nutrient interaction [$F(1,22) = 16.86, P < 0.01$]. Post hoc analysis revealed that intake of maltodextrin was significantly greater than intake of corn oil only for rats that had been poisoned after IG corn oil infusions. This result indicated that IG maltodextrin poisoning was able to attenuate the apparent strong preference for the carbohydrate solution. This result also demonstrates that the learned association between LiCl and the orally detected components of these stimuli was maintained in the absence of the poison during the flavor-nutrient training. Summation test. Figure 8 shows data from the 30-min two-bottle summation tests comparing intake of the poisoned infusate (X), this infusate mixed with its associated flavor (XA), and this infusate mixed with its nonassociated flavor (XB). Figure 8 shows intakes of XA and XB in their separate comparison tests with X. Intake of X shown is mean intake across both X vs. XA and X vs. XB tests. Intake of X did not differ across tests, nor was it dependent on whether X was the corn oil or the maltodextrin solution (largest $F = 4.33, P > 0.05$). An ANOVA on these data yielded a main effect of nutrient [$F(1,18) = 31.24, P < 0.01$] with more maltodextrin being consumed than corn oil overall, and a main effect of solution type (X vs. XA vs. XB) [$F(2,36) = 35.63, P < 0.01$]. This latter effect was further analyzed, and it was found that intake of XA and XB were both significantly greater than intake of X and that intake of XB was also significantly greater than XA. None of the variables in the ANOVA were found to interact with poisoned nutrient type (largest $F = 2.95, P > 0.05$), indicating that the summation effect was not dependent on which nutrient was poisoned. It may be that consumption of the poisoned infusate was increased when mixed with either its associated on nonassociated flavor because adding the flavors increased the palatability of the infusate. However, differences in palatability per se cannot explain greater consumption when the poisoned infusate was mixed with its nonassociated compared with its associated flavor. Rather, this difference suggests that the nonassociated flavor reduced the intake-suppressive effects of the poisoned infusate more than did the associated flavor, based on differences in what was learned during training.

In summary, the results of this experiment indicated that the macronutrient infusions seem to be equally effective, whether they were previously poisoned or not poisoned, at increasing the oral preference for arbitrary nonnutritive flavors. Furthermore, the same pattern of preference shifts was seen when the infusate aversion training preceded the electronic esophagus flavor-nutrient training (the present results) as when the aversion training followed the flavor-nutrient conditioning (experiment 1). However, data from the summation tests in which the CS flavors were mixed together with the poisoned infusate indicated that some learning did occur that was dependent on nutrient poisoning. Specifically, animals indicated a preference
for the compound containing the flavor paired with the non-poisoned nutrient over the compound containing the flavor paired with the poisoned nutrient. Furthermore, we were able to replicate the main result of experiment 2, again demonstrating that animals are capable of learning about cues produced by IG infusion of different nutrients and are capable of expressing that learning by altering their preference behavior when allowed to orally consume those nutrients for the first time.

**EXPERIMENT 3b**

One possible explanation for the finding in experiments 2 and 3a, that association between an IG nutrient stimulus and LiCl alters preference for those same stimuli when they are presented orally, is reflux. That is, after being infused into the stomach, these stimuli could hypothetically make their way up the esophagus and into the mouth, allowing the animal to taste these stimuli during the training procedure. If this were the case, then the intestinal taste aversion phenomenon that has been demonstrated here would constitute nothing more than a simple oral taste aversion. The summation test in experiment 3a provides some evidence against this hypothesis. The results of that test showed that the presence of the flavor CS that had been associated with the poisoned IG nutrient suppressed intake of the poisoned IG nutrient more than did the presence of the flavor CS that had been associated with the nonpoisoned nutrient. However, these flavor CSs had been trained to signal the appetitive UCS produced by IG infusion. Neither flavor was present during aversion training, the time when learning based on gastric reflux would presumably occur. Thus differences in the ability of each CS to promote intake during the summation test would have to be the result of differences in their association with the appetitive postdigestive UCS produced by the infusions. There is no reason to expect that differences in reflux between infusates would occur during the original appetitive training phase of the experiment. Thus there seems to be no way to explain the results of the summation test with reference to reflux.

The purpose of experiment 3b was to provide a more direct assessment of the reflux hypothesis. In this study, animals were trained using the same aversion procedure described for experiment 3a. However, in experiment 3b, the IG infusates were the same nonnutritive stimuli (i.e., lemon-lime- and orange-flavored saccharin solutions) that were used in experiment 2 to verify the efficacy of the LiCl. Thus it is clear that these stimuli are distinguishable when presented to rats orally. If LiCl injection produces reflux of these infusates, then the rats should be able to taste these flavors and associate them with illness. Accordingly, the rats should exhibit a preference for their nonpoisoned compared with their poisoned infusate when given the opportunity to orally ingest each substance. On the other hand, no preference between the poisoned and nonpoisoned infusates would make the hypothesis that the rats tasted the infusates during aversion training less plausible. Rather this outcome would bolster the idea that IG infusates differ in the extent to which they give rise to distinct nutritive postoral sensory consequences that can be associated with other events and that carbohydrates and fats may serve as more salient stimuli than the nonnutritive flavors.

**Method**

**Subjects.** Subjects were the same as those used in experiment 3a.

**Stimuli.** Stimuli were 0.2% saccharin solutions flavored with 0.05% lemon-lime- or orange-flavored Kool-Aid.

**Procedure.** As in experiment 3a, animals were given four 20-min training sessions. During two of these sessions, they received IG infusions of either the lemon-lime- or the orange-flavored saccharin solution using the same infusion parameters as for the maltodextrin and corn oil in experiment 3a. In the two alternating sessions, they received infusions of the opposite flavored solution. No oral stimuli were provided for the animals to consume during these sessions. Fifteen minutes after each session, all animals were given an intraperitoneal injection of either saline or LiCl. Animals were divided into two equal groups, counterbalanced with respect to the nutrient that had been poisoned in experiment 3a. For one group of animals, the lemon-lime solution was paired with LiCl and the orange solution with saline. The remaining animals were given the opposite pairings. The aversion training sessions were conducted under the same conditions as in experiment 3a. After this training, a two-bottle test session was conducted in the home cage in which the orange and lemon-lime solutions were presented simultaneously with no IG infusions, and intake of the solutions was measured at 30 min and 1, 2, and 4 h.

**Results and Discussion**

It can be seen in Fig. 9 that intake of the two flavored solutions did not differ during the first 30 min of the test session in which the two flavored saccharin solutions were presented orally. This observation was confirmed by an ANOVA that yielded no effect of poisoning and no significant interactions with poisoned flavor or previously poisoned nutrient (largest $F = 0.22, P < 0.05$). The low level of intake at 30 min leaves open the possibility that the failure to observe an aversion effect is due to a floor effect. However, this concern is reduced by the fact that there was no significant difference

![Fig. 9. Mean intake of orally presented noncaloric flavor solutions in a 30-min two-bottle choice test after pairing IG infusion of one flavor with intraperitoneal LiCl injection (poisoned) and the other with intraperitoneal saline injection (nonpoisoned).](http://ajpregu.physiology.org/doi/10.1152/ajpregu.011005.2004)
between intake of the poisoned and nonpoisoned flavors at any time point, although cumulative intake climbed to a mean of 37.4 g over 4 h (poisoned = 34.7 ± 5.0 g, nonpoisoned = 40.0 ± 4.4 g).

The present result indicates that distinctly flavored but noncaloric stimuli presented IG are ineffective CS in an aversion paradigm under training and test conditions similar to those that produced an aversion using a fat or carbohydrate solution. Because, as experiment 2 illustrated, these noncaloric stimuli sampled by mouth can be readily conditioned by LiCl poisoning, the lack of conditioning in the present study strongly suggests that the nonnutritive CSs were not stimulating the oropharynx by reflux. This result also suggests that olfactory cues were not serving as the discriminative stimuli, nor is it likely that rats were contacting the infused stimuli leaking from the catheter or other unintended exposure during the procedure. The following experiments provide additional evidence against these types of hypotheses.

**EXPERIMENT 4: DUODENAL INFUSIONS IMPLICATE INTESTINAL CHEMORECEPTORS IN LEARNING ABOUT INTESTINAL TASTES**

IG infusions were used in the first three experiments, in part to provide comparability with the earlier electronic esophagus literature. Because gastric afferents seem to be predominately mechanoreceptors, whereas intestinal afferents are predominately chemoreceptors (23), the observed macronutrient discriminations presumably depend on the emptying of the infusates into the intestine. In the present experiment, two additional tests were executed using duodenal catheters to evaluate whether the CSs operated postgastrically (experiment 4a) and whether reflux or inadvertent leakage or spillage of the infused stimuli was responsible for the conditioning observed (experiments 4a and 4b).

**EXPERIMENT 4a**

To verify directly that the small intestine mediates the effects of intestinal taste aversion training or oral taste preference, we compared the efficacies of IG and intraduodenal infusions in an additional group of rats. This experiment also provided additional evidence that the conditioning effect was not the result of reflux of gastric contents.

**Methods**

Subjects. Subjects were 32 naive rats of the same description and maintained under the same conditions as experiment 1.

Surgery and apparatus. Sixteen rats underwent the same surgical procedures for implantation of gastric cannulas as described in experiment 1. The remaining 16 rats underwent a similar procedure, but after being inserted into the stomach the cannula was advanced through the pyloric sphincter and anchored to the luminal duodenal wall 4 cm distal to the pyloric sphincter. The rats were trained and tested using the same apparatus as described in experiment 1.

Stimuli. Oral and IG/intraduodenal stimuli were the same as those used in experiment 1.

Procedure. After recovery from surgery, the rats were reduced to 85% of their ad libitum postsurgical body weights. They then received two 30-min habituation sessions in which they were placed in the conditioning chamber and attached to the apparatus, but no oral or IG stimuli were presented. The rats were then given four aversion-training sessions, in which they were placed in the infusion cages for 20 min and either 7.1% corn oil emulsion or 16% maltodextrin (2 sessions each, alternating) was delivered IG or intraduodenally at a rate of 0.6 ml/min in a repeating cycle of 10 s on/10 s off for a total infusion volume of 6 ml. For half of the animals (gastric catheter n = 8; duodenal catheter n = 8), after the sessions in which they received IG/intraduodenal maltodextrin, they were given an intraperitoneal injection of LiCl and given an intraperitoneal injection of saline after the session in which they received IG/intraduodenal corn oil. The remaining rats received the reverse nutrient-drug contingency (corn oil-LiCl, maltodextrin-saline).

After completion of this training phase, the rats were given a 4-h two-bottle preference test between the corn oil and maltodextrin solutions in the home cage. Intake of the nutrient solutions was measured at 30 min and 1, 2, and 4 h.

**Results and Discussion**

The results from the first 30 min of the two-bottle infusate intake test for animals implanted with either gastric (left) or duodenal (right) catheters are shown in Fig. 10. Mean intake of each infusate for the poisoned and nonpoisoned treatment conditions, along with statistical analysis, is presented in the figure legend. There was no difference in intake of the nutrient solutions between the two catheter placements, and both groups of animals consumed greater amounts of the nonpoisoned solution than the poisoned. Both of these observations were confirmed statistically, as there was a main effect of poisoning [F(1,28) = 12.79, P < 0.01], but no significant effects involving catheter type [largest F(1,28) = 0.26]. Consistent with previous results, there was also an interaction between poisoned nutrient type and poisoning [F(1,28) = 9.33,
stimuli employed as GI CSs were insufficiently distinctive for any conditioning when flavored nonnutritive solutions that readily support oral taste aversion conditioning were infused into the stomach and were thus the solutions that would have leaked from the catheter. It is arguable, however, that experiment 3b was inconclusive either because the two nonnutritive stimuli employed as GI CSs were insufficiently distinctive for optimal oral discrimination or because the animals had previously been trained in experiment 3a and thus preconditioning experience might have minimized efficacy of LiCl as a UCS.

To address these hypothetical concerns and to provide an additional control for reflux through the catheter, the present experiment 1) used groups of naive animals that had not previously been conditioned, thus eliminating any issue of preexposure; and 2) introduced several variations into the training paradigm. In particular, the last fractions of the nutritive CSs (corn oil and maltodextrin) were infused directly into the duodenum bypassing the stomach. This suggests that the detection of these solutions is likely taking place postgastrically and, furthermore, reduces the possibility that this effect is attributable to reflux of the nutrient solutions into the oral cavity during aversion training by introducing stimuli beyond the pyloric sphincter and eliminating potential confounds present in the previous experiment (i.e., preexposure to LiCl and the use of nonnutritive infusates).

**EXPERIMENT 4b**

Both experiments 3b and 4a indicated that the conditioning we have observed is not the result of the infused nutrients moving by reflux back up the GI tract to produce oral stimulation. Conceivably, though, if any infusate were to leak at the external tip of the catheter when the animal was detached from the infusion line, this could make the infusate accessible so that the animal might sample the solution by grooming or licking the site where the catheter was externalized. Experiment 3b argues that such a scenario is unlikely to account for the observed conditioning, because the animals did not evidence any conditioning when flavored nonnutritive solutions that readily support oral taste aversion conditioning were infused into the stomach and were thus the solutions that would have leaked from the catheter. It is arguable, however, that experiment 3b was inconclusive either because the two nonnutritive stimuli employed as GI CSs were insufficiently distinctive for optimal oral discrimination or because the animals had previously been trained in experiment 3a and thus preconditioning experience might have minimized efficacy of LiCl as a UCS.

Finally, the animals were not disconnected from the infusion lines until 15 min after the flavored saccharin flush of the catheter to provide additional certainty that any backpressure associated with the infusions would have dissipated.

**Method**

**Subjects.** Subjects were 32 naive animals of the same description and maintained under the same conditions as in experiment 1.

**Surgery and apparatus.** Animals were implanted with duodenal cannulas as described in experiment 4a; they otherwise underwent the same surgical procedures and were trained and tested using the same apparatus as described for previous experiments.

**Stimuli.** Stimuli were the same solutions described in experiment 1.

**Procedure.** After recovery from surgery, the rats were reduced to 85% of their ad libitum postsurgical body weights. They then received two 30-min habituation sessions on successive days in which they were placed in the conditioning chamber and attached to the apparatus, but no oral or intraduodenal stimuli were presented.

Animals were then given four 35-min training sessions (2 poisoned trials; 2 nonpoisoned) over four successive days. During the first 20 min of each session, animals were intraduodenally infused with either 7.1% corn oil (2 sessions) or 16% maltodextrin (2 sessions) at a rate of 0.6 ml/min in a repeating cycle of 10 s on/10 s off for a total infusion volume of 6 ml (same parameters as experiment 4a). After this nutrient infusion, and without detaching the infusion lines from the catheters, animals received an additional 1 ml infusion of either a grape-flavored (2 sessions) or cherry-flavored (2 sessions) saccharin solution using the same rate parameters. This saccharin infusion cleared the infusion line (dead space volume = 0.32 ml) and the lumen of the catheter (volume = 0.07 ml) of nutrients to ensure that any taint that might leak when the animals were detached would be the flavored saccharin. The animals then remained in the conditioning chamber for an additional 15 min with the infusion line attached to the catheter. The particular flavor of the saccharin solution was consistently paired with one nutrient, such that animals always received corn oil followed by cherry and maltodextrin followed by grape or vice versa. Nutrient-flavor pairings and the order in which nutrient-flavor pairings were presented were counterbalanced. At the conclusion of each session, animals were removed from the conditioning chamber, the cannula and backmount areas were blotted with paper towels as further precaution against any inadvertent leakage, and cannulas were flushed with ~0.5 ml saline. Animals then received an intraperitoneal injection of either LiCl or saline. For half the animals, the LiCl injections followed infusions with the corn oil emulsion, while saline injections followed the maltodextrin infusions. The remaining animals received the opposite nutrient-poisoning contingency. Similarly, half the animals received LiCl after infusion of the grape solution and saline after the cherry solution, while the other half received the opposite pairing.

On the 2 days after the completion of training, two-bottle test sessions were conducted in the home cage. On the first day, all animals received one bottle containing the corn oil emulsion and one bottle containing the maltodextrin solution. On the
second day, all animals received one bottle containing the grape-flavored solution and one bottle containing the cherry-flavored solution. Intake of these solutions was recorded at 30 min, 90 min, and 4 h.

Results and Discussion

One animal became detached from the infusion apparatus during a training session and could conceivably have consumed infusate during training; therefore the data from this animal were discarded from the analysis. Figure 11 shows the intake of the poisoned and nonpoisoned nutrient solutions during the first 30 min of the two-bottle intake test. Figure 12 shows the intake of the poisoned and nonpoisoned nonnutritive flavor solutions during the same period. Mean intake of each infusate for the poisoned and nonpoisoned treatment conditions, along with statistical analysis, is presented in the figure legend.

The data in these figures are consistent with the results of our previous experiments. That is, the animals learned to distinguish the two nutrient solutions that had been presented intraduodenally and reduce their consumption, on first oral contact, of the nutrient paired with LiCl. However, as in experiment 3b, the animals did not show any learning about the two nonnutritive, but distinctly flavored, solutions based on their pairing with LiCl. An ANOVA looking at the nutrient intake data yielded a main effect of poisoning \( F(1,29) = 4.51, P < 0.05 \), demonstrating reliably lower intake of the poisoned compared with the nonpoisoned nutrient. There was also a significant poisoning \( \times \) poisoned nutrient type interaction \( F(1, 29) = 25.11, P < 0.01 \), again due to the overall greater intake of the maltodextrin solution regardless of which nutrient had been poisoned.

Analysis of the results of the nonnutritive flavor intake test yielded no significant effects, confirming that there was no difference in intake between the flavor infusion paired with LiCl and that paired with saline (see Fig. 12). As in experiment 3b, intake of the flavored saccharin solutions during this test was quite low, and the failure to see intake differences between the poisoned and nonpoisoned flavors could be attributed to a floor effect. However, again, despite increased intakes over 4 h of testing, cumulative intakes of the two flavors still did not statistically differ from one another (poisoned = 13.6 ± 1.7 ml, nonpoisoned = 12.9 ± 1.9 ml).

GENERAL DISCUSSION

In agreement with earlier findings (19), our data from experiment 1 showed clearly that the appetitive UCSs produced, respectively, by IG maltodextrin and IG corn oil were sufficient to establish conditioned flavor preferences. Yet, associating these IG nutrients with malaise appeared to have little impact on their ability to either maintain previously learned flavor preferences (experiment 2) or to promote learning of preferences (experiment 3a) for flavor cues. Surprisingly, although pairing IG nutrient infusions with illness failed to alter intake of flavor cues that were associated with these infusions, we found that such pairings significantly reduced intake of the nutritive GI (used here to refer to either IG or intraduodenal) infusions when they were first made available for oral consumption. That is, in experiments 2, 3a, 4a, and 4b, pairing GI infusion of nutrients (maltodextrin, corn oil) with injection of LiCl reduced subsequent oral consumption of these nutrients compared with when these GI infusions had been followed by injection of saline.

One way to explain why our rats showed less oral consumption of poisoned compared with nonpoisoned nutritive GI infuses is to suggest that the rats were somehow able to taste by mouth the IG-infused nutrient solutions during aversion training. If this were the case, differential oral intake of the poisoned and nonpoisoned GI solutions could be characterized as simple taste aversion.

This type of simple taste aversion learning could occur if malaise induced reflux of some of the IG infuses into the oral cavity, thereby permitting oropharyngeal stimulation produced by the infusate to be associated directly with an aversive IG stimulation produced by the LiCl UCS. Despite the widely
accepted view that rats do not exhibit emesis and are thus incapable of this type of reflux, we subjected this potential explanation of our findings to direct experimental analysis.

In experiment 3b we found that pairing GI infusions of nonnutritive, but distinctly flavored, test solutions with injection of either LiCl or saline failed to alter intake when the nonnutritive infusates were first presented for oral consumption. That is, oral intake of these nonnutritive infusates did not depend on whether they had been paired with illness when they were infused IG. If reflux was the basis for associating the taste of IG maltodextrin or corn oil with illness, why reflux failed to result in similar conditioning of distinctively flavored nonnutritive solutions is unclear. The fact that robust taste aversion was observed in experiment 2 when these same nonnutritive stimuli were trained with LiCl and saline suggests that the rats had little difficulty discriminating between these flavors when they were presented orally. One could speculate that reflux somehow reduces the discriminability of nonnutritive cues more than nutritive orosensory stimuli or that less reflux occurs with nonnutritive compared with nutritive infusates. However, there appears to be no evidence to support this type of speculation, which is not surprising given the absence of compelling evidence that gastric reflux of any type occurs in rats.

The results of experiment 4a addressed the possibility of reflux in another way. This study showed that reductions in oral preference for nutrients that were paired with illness could be obtained when the nutrients were infused intestinally. Under these conditions, the opportunity for reflux of infusates into the oropharyngeal cavity would be greatly reduced compared with gastric infusion of nutrients as in experiments 1–3b. Nonetheless, on first oral contact, rats trained with intestinal infusions exhibited clear preferences for nonpoisoned compared with poisoned infusates. Experiment 4a showed that magnitude of these preferences did not depend on whether gastric or intestinal infusions were performed during aversion training. This latter finding is of special importance because while both gastric and intestinal infusions involve detection of nutrients by intestinal receptors, the likelihood of contact by nutrients with oropharyngeal receptors would be much greater after gastric than intestinal infusions. This difference, considered with the finding that both types of infusions produced similar patterns of oral preference, provides evidence that preference was based on detection of intestinal rather than oropharyngeal stimulation.

In experiments 1–4a, we attempted to prevent inadvertent oral contact with infusates by wiping away any infusate that might have dripped or leaked on to the rats when they were detached from the infusion apparatus. Although we had no reason to suspect that this procedure failed to prevent direct oral contact with the GI infusates, in experiment 4b we took additional steps to discount this possibility. Specifically, any nutritive infusate that was present at the external catheter tip at the end of a training session was flushed into the intestine before the catheters were disconnected. In addition, the evacuated nutritive infusates were replaced in the catheter with distinctly flavored, nonnutritive solutions. Thus, if any inadvertent leakage from the external catheter tip occurred when the catheter was removed, it would be flavored nonnutritive solution rather than a nutritive infusate. Furthermore, as a means of preventing backpressure from forcing infused nutrients from the intestine back up the catheter, 15 min (ample time for any backpressure to dissipate) was allowed to elapse between the end of the nutrient infusions and when the catheters were detached.

With these procedures there was clearly much less opportunity for direct oral contact with leaked or spilled nutritive infusates than with the flavored nonnutritive solutions. Nonetheless, the results of experiment 4b showed that pairing with LiCl altered subsequent oral preference for the nutritive intestinal infusates but not for the flavored nonnutritive solutions. These findings indicate that preference conditioning was based on learning about intestinal stimulation, rather than learning based on oral contact with infusates that might have been leaked or spilled inadvertently during training.

In summary, our findings provide no evidence that GI infusates made direct contact with oropharyngeal receptors during intestinal taste aversion training or that such contact was the basis for preferences observed when our rats were allowed to consume GI infusates by mouth during subsequent testing. Thus consideration must be given to other ways in which pairing GI nutrient infusion with malaise induced by LiCl with could interfere with subsequent oral intake of the poisoned nutrient.

One possibility is that GI infusates made contact with oropharyngeal receptors, not directly, but postabsorptively via the bloodstream. Reports that humans can taste intravenous infusions of saccharin (8) or medicinal compounds (7) have suggested that substances that can enter the bloodstream may make contact with oral receptors and in this way convey “taste” information without entering the oral cavity in the normal fashion. In fact, it has been shown that an intravenous saccharin solution can attenuate neophobia to oral intake of a more dilute saccharin solution and that intraperitoneal saccharin paired with LiCl can induce an aversion to an orally consumed more dilute saccharin solution (2). It may be that such “vascular taste” stimulation can provide an explanation for the present results. However, other considerations seem to pose problems for this interpretation. Results from prior studies indicated that a longer delay (i.e., 2 h) and/or a more dilute solution (presumably matching the concentration of “flavor” in the bloodstream) is required to produce a significant aversion (2). Considering that in the present studies significant aversion was obtained with a delay of only 15 min and that the training and test solutions were of the same concentration, this explanation seems unlikely. Furthermore, the corn oil infusate would be expected to enter the circulation quite slowly, thus making detection via this type of postabsorptive mechanism particularly unlikely. Our findings (e.g., experiments 2 and 3a) that pairing infusions of corn oil with illness reduced intake of corn oil compared with when corn oil infusions were paired with saline are also problematic for intravascular taste interpretations.

Another hypothesis is that oral preference for nutritive infusates depended on GI sensing at the time of the preference tests. At some point after ingestion, maltodextrin and corn oil presumably stimulate the same types of GI receptors that were stimulated during GI infusions of these nutrients. This raises the possibility that the pattern of oral preference exhibited by our rats when they were offered maltodextrin and corn oil by mouth was based solely on the subsequent contact by these nutrients with GI receptors like those that were stimulated during GI infusions. However, the results of both experiment 2
and experiment 3a disagree with this hypothesis. In experiment 2, intake of previously trained nonnutritive flavor cues was unaffected by whether ingestion of those flavors was accompanied by IG infusion of the previously poisoned or nonpoisoned nutrient, while in experiment 3a, flavor-nutrient conditioning with IG nutrients was not altered by prior poisoning of either the IG maltodextrin or corn oil. In contrast, the rats in both these experiments showed reduced oral preference for the nutritive infusate that had been paired with malaise during IG aversion training. These results indicate that oral preference was not based solely on concomitant detection in the gut of a previously poisoned or nonpoisoned infusate but depended on oropharyngeal sensing of the properties of the infusates at the time of testing.

In fact, these results also bring into question whether GI stimuli produced by IG nutrient infusions were actually associated with the aversive UCS produced by LiCl. If the appetitive postigestive UCS produced by an IG nutrient infusion had been associated with malaise, presentation of the flavor that was associated with that IG nutrient would be expected to excite the representation of illness via activation of the IG nutrient-illness association. Excitement of the memory of illness would then be expected to reduce preference for that flavor. Our failure to obtain this outcome cannot be attributed to lack of association between the flavor CS and the IG nutrient cues. Evidence for this type of association formation was provided in experiment 1 by the finding that our rats exhibited significant preference for flavors that were associated with IG maltodextrin relative to flavors paired with corn oil. Neither can the result be attributed to a weak or nonexistent illness UCS because this same UCS promoted strong taste aversion learning after oral intake of flavored solutions.

Based on the above considerations, the main challenge presented by our findings is to describe how pairing with LiCl could alter oral preference for gastrically or intestinally infused nutrients without prior direct or indirect contact between these nutrients and oropharyngeal receptors at the time of training and without clear evidence of a strong association between nutrient-specific GI stimulation and malaise. We offer the following speculative hypothesis. The sensory consequences of maltodextrin, corn oil, and perhaps other nutrient sources may be coded orally even when they are detected only by receptors in the gut. If this were the case, IG infusions of a nutrient followed by injection of LiCl could permit illness to be associated with certain orosensory properties of the nutrient without the nutrient coming into direct contact with oral receptors. For example, in this type of system, GI infusion of maltodextrin might activate mechanisms involved with the central encoding of both the oral and postoral stimulus properties of carbohydrate. Although illness might not be strongly associated with postoral stimuli produced by nutrient infusion, it could be strongly associated with the central representation of the orosensory features of carbohydrate. Then, when that central representation is subsequently excited by oral intake of carbohydrate, consumption would be reduced based on the prior association of that representation with illness.

Consistent with this perspective, several observations suggest that the conventional distinction between oral and postoral receptors in the GI tract is arbitrary and that extensive central integration of the respective afferent inflows occurs. Gustatory receptors of the oropharynx are innervated by the facial nerve, glossopharyngeal nerve, and vagus, and these three cranial nerves carry similar primary taste information, although in different proportions. The vagus provides much of the innervation of the receptor sheet of the proximal GI tract as well, and electrophysiological evidence suggests that its abdominal branches carry similar chemoreceptor information from the gut (16, 20), although again perhaps in different proportions. Vagal afferents are associated with cells in the small intestine that have the morphological features of “taste cells” (13, 21).

In addition, both gustatory receptors in the mouth and chemoreceptors in the intestine express the taste-specific G protein α-gustducin (13, 17). Embryologically, the afferents of the mouth and the GI tract are part of a common visceral nervous system (12). Furthermore, the gustatory neurons of the oropharynx and the chemoreceptive afferents of the gut share a common second-order relay nucleus (i.e., nucleus of the solitary tract). The chemoreceptor inputs from the mouth and GI tract also share common third-, fourth-, and higher-order relay stations in the central nervous system. Although substantial segregation of the inputs occurs within the nuclei and pathways of the visceral neuroaxis, the fact that all chemoreceptor information is processed in common relay nuclei implies that there is potentially considerable integration of the information. Thus these common neural pathways may allow for a translational system by which information gained through GI chemoreceptors can be processed and converted into information that is later accessible to be compared with new information coming from oral gustatory receptors (see also Ref. 16). This oral taste sensation could then be assessed based on prior learning about the postoral effects of the same or similar foods previously encountered.

It remains unclear from the results reported here, as well as other studies using different macronutrient infusates (3, 9, 19), which specific properties of the infusates might be detected, integrated into the infusate-illness association, and centrally encoded to allow for oral determination of intake and selection. The two nutrient infusates we used differed along a number of dimensions that are detectable by the GI tract. While they were equated in caloric density, in the corn oil emulsion, 100% of those calories were in the form of fat, while all calories in the maltodextrin solution were in the form of carbohydrate. The two solutions had similar pH values and very low viscosities; however, there are other factors, such as osmolarity, which were not controlled for and likely differed between the two solutions. One direction for future work on this phenomenon may focus on matching solutions on this and other physicochemical properties to identify what is being used, both orally and postorally, to discriminate between these solutions.

The issue of whether the gastrically and intestinally infused nutrients in the present experiments serve as preabsorptive or postabsorptive CSs, as mentioned above, is another direction for future work. As discussed in experiment 3b, several arguments favor an intestinal chemoreceptive or preabsorptive site of action for the stimuli. The above discussion of GI chemoreceptors is also consistent with such a preabsorptive mechanism. On the other hand, the preference learning phenomenon demonstrated with the conventional electronic esophageus paradigm seems, on the basis of denervation experiments (18, 25, 26), to have a postabsorptive basis. Additional experiments are needed to assess whether the intestinal taste aversion phenom-
enon described in the present series of studies involves preabsorptive afferent detection or postabsorptive sensing.

The role of macronutrient content has previously been considered in the development of sensory-specific satiety (15); however, more recently, this question has been addressed using a gastric or intestinal infusion technique in an attempt to rule out orosensory factors. Foster and colleagues (9) found that a gastric or intestinal infusion of fat suppressed oral intake of the same fat out orosensory factors. Burggraf and coworkers (3) found that an IG duodenal infusion of fat suppressed oral intake of the same fat out orosensory factors. Foster and colleagues (9) found that a gastric or intestinal infusion technique in an attempt to rule however, more recently, this question has been addressed using their macronutrient composition.

In summary, the experiments presented here demonstrate that rats are capable of detecting postoral cues produced by corn oil and maltodextrin infusions, differentiating between the two infusates based on these cues and selectively associating one set of cues with GI illness. Furthermore, rats are able to translate this postoral information into something that allowed them to determine which of these infusates was poisoned when they were first presented for oral consumption. This suggests a correspondence, presumably centrally mediated, between GI and oropharyngeal sensory information. Exploration of this correspondence may have significant implications for describing the function and capability of the GI tract and for understanding the larger role of orosensory and postoral stimuli in the control of food intake and body weight regulation.

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