Am J Physiol Regul Integr Comp Physiol 302: R1351–R1363, 2012.
First published March 14, 2012; doi:10.1152/ajpregu.00702.2011.

Rapid stimulus-bound suppression of intake in response to an intraduodenal nonnutritive sweetener after training with nutritive sugars predicting malaise

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Submitted 5 January 2012; accepted in final form 12 March 2012

Schier LA, Davidson TL, Powley TL. Rapid stimulus-bound suppression of intake in response to an intraduodenal nonnutritive sweetener after training with nutritive sugars predicting malaise. *Am J Physiol Regul Integr Comp Physiol* 302: R1351–R1363, 2012. First published March 14, 2012; doi:10.1152/ajpregu.00702.2011.—In a previous report (Schier et al., *Am J Physiol Regul Integr Comp Physiol* 301: R1557–R1568, 2011), we demonstrated with a new behavioral procedure that rats exhibit stimulus-bound suppression of intake in response to an intraduodenal (ID) bitter tastant predicting subsequent malaise. With the use of the same modified taste aversion procedure, the present experiments evaluated whether the sweet taste properties of ID stimuli are likewise detected and encoded. Thirsty rats licked at sipper spouts for hypotonic NaCl for 30 min and received brief (first 6 min) yoked ID infusions of either the same NaCl or an isomolar lithium chloride (LiCl) solution in each session. An intestinal taste cue was mixed directly into the LiCl infusate for aversion training. Results showed that rats failed to detect intestinal sweet taste alone (20 mM Sucralose) but clearly suppressed licking in response to a nutritive sweet taste stimulus (234 mM sucrose) in the intestine that had been repeatedly paired with LiCl. Rats trained with ID sucrose in LiCl subsequently generalized responding to ID Sucralose alone at test. Replicating this, rats trained with ID Sucralose in compound with 80 mM Polycose rapidly suppressed licking to the 20 mM Sucralose alone in a later test. Furthermore, ID sweet taste signaling did not support the rapid negative feedback of sucrose or Polycose on intake when their digestion and transport were blocked. Together, these results suggest that other signaling pathways and/or transporters engaged by caloric carbohydrate stimuli potentiate detection of sweet taste signals in the intestine.

gastrointestinal; taste receptor; preabsorptive signals; artificial sweetener; food learning

THE DISCOVERIES that putative chemoreceptive cells in the epithelium of the gastrointestinal (GI) tract express taste receptor molecules and intracellular signaling proteins (e.g., 3, 4, 10, 17–19, 22, 27, 28, 48, 53) as well as exhibit morphological features resembling those of taste cells on the tongue (e.g., 12, 18) have stimulated interest in how preabsorptive food-related signals arising from the lumen of the GI tract are transduced to affect digestion and ingestion (see, for example, the series of review articles entitled "Nutrient Tasting and Signaling Mechanisms in the Gut" published in *American Journal of Physiology*: Refs. 5, 13, 20, 32, 41). To address such questions, we recently developed and employed a new behavioral paradigm to demonstrate that a bitter tastant in the duodenum produces stimulus-bound reductions in ongoing ingestion (37). The present set of experiments addresses similar functional questions using the same behavioral paradigm to evaluate the effects of sweet tastants in the duodenum.

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Sweet taste receptors (T1R2 and T1R3) and the associated signaling protein α-gustducin are localized in the epithelium of the postoral GI tract (10, 17, 19, 27, 28, 47, 48). Nutritive and nonnutritive sweet tastants delivered directly to the GI tract in combination with glucose elicit rapid insertion of GLUT-2s to the epithelial surface to increase glucose absorption, and when given as part of a low carbohydrate diet (or to a fed subject), increase lumenal SGLT1 expression to accommodate higher glucose loads over the long term (27, 28, 46). T1Rs are further implicated in local glucoregulation by their association with the incretin-releasing L/K cells (19, 23). Glucose in the GI tract stimulates the release of GLP-1 from these enteroendocrine cells, a response that is severely blunted in α-gustducin knockout and T1R3 knockout mice (19, 23). Beyond such roles in the local physiology of the gut, little is known about postoral sweet taste signaling, especially with respect to ingestive behavior. But given oral sweet taste signaling is a critical determinant in the control of food intake, and GLP-1 released from GI cells is additionally integrated into satiation (34), determining whether sweet taste information is encoded in GI feedback for ingestive behavior may reveal additional functional links between GI sweet taste, metabolic function, and body weight regulation.

Oral sweet receptors (T1R2+3) have a high affinity for many of the simple carbohydrates, such as sucrose (30). Postorally, these same compounds reinforce conditioned flavor preferences and stimulate the release of satiety signals to negatively feedback on ingestion (36, 39, 40), but whether the sweet taste property of these nutritive compounds is detected at GI preabsorptive receptors and critically encoded for these processes is unknown. Previously, a handful of studies have shown that sweet-blind mice (T1R3, TRPM-5, or α-gustducin knockouts) still develop a preference for an oral flavor or spatial location associated with intragastric or normally consumed sucrose or glucose (7, 33, 40). Meanwhile, normal mice fail to increase consumption of an oral flavor associated with intragastric infusions of a sweet tasting, but metabolically inert, solution (Sucralose) (40). Together these studies suggest that GI sweet taste signaling is not necessary for conditioned flavor preference, but they do not altogether rule out the possibility that sweet tastes are detected and transduced from a postoral receptor site under normal conditions or for other functions. For one thing, these conditioned flavor preference experiments have used extended training schedules (i.e., exposure to the contingencies 22–24 h/day). With this access, sweet-blind mice may compensate by associating the flavor or spatial location with other rewarding postingestive properties of sucrose (i.e., delayed metabolic effects, but also possibly alternative preabsorptive sensory effects), and, for normal mice, the rewarding properties of IG Sucralose may extinguish over that extended exposure in the absence of the expected caloric effects. Beyond this, it may be that GI sweet taste signals are integrated for

sensory-discriminative functions but not through the reward pathways underlying conditioned flavor preferences.

In Schier et al. (37), we developed a behavioral paradigm with the capacity to examine the detection and discrimination of early preabsorptive taste-like signals arising from the intestine and their effects on ongoing ingestive behavior, separate from any delayed postabsorptive or rewarding effects of the same stimuli. In this approach, a modified conditioned taste aversion, thirsty rats lick at sipper spouts for hypotonic NaCl for 30 min each day and receive brief (first 6 min) yoked intraduodenal (ID) infusions of either the same NaCl solution or an isomolar lithium chloride (LiCl) solution. LiCl infusions produce a mild malaise ~15 min after the infusion. Because the solution at the sipper spout is consistent across both of these session types, rats are unable to use oral cues to predict LiCl malaise and must instead use intestinal cues to solve the discrimination. For instance, as shown in Fig. 1, when a bitter taste cue denatonium benzoate (DB, 10 mM) was selectively added to the LiCl infusate to condition an intestinal taste aversion, rats readily learned to rapidly, but transiently, inhibit ongoing intake upon the arrival of the bitter stimulus to the intestine (Ref. 37, *experiments 1* and *2*).

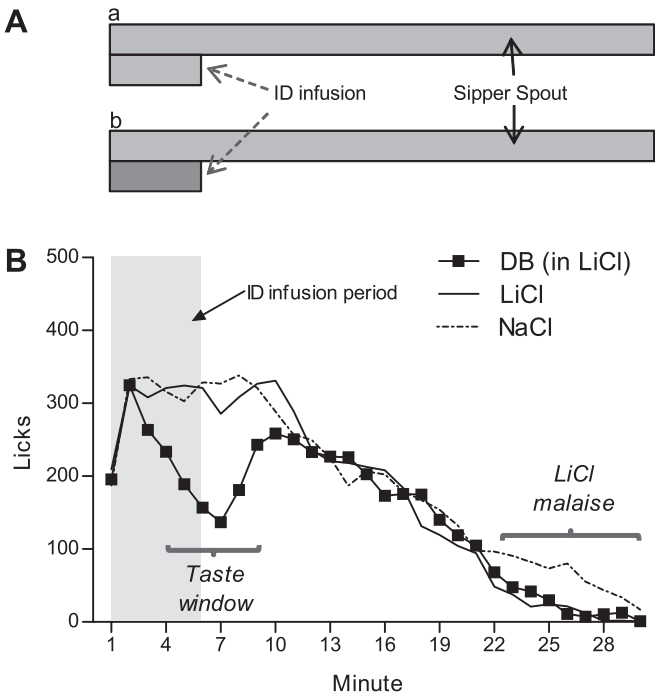

Fig. 1. *A*: for intestinal aversion training, rats licked for 0.12 M NaCl at the sipper spout for 30 min (long light gray rectangle) on each daily session. On half of the probe sessions rats receive a yoked infusion of 0.12 M NaCl vehicle infusate during the first 6 min (short light gray rectangle, *a*), whereas on the other half of the probe sessions, rats receive a yoked infusion of an isomolar LiCl vehicle (short dark gray rectangle, *b*). To condition intestinal taste discriminations, an intestinal taste cue (e.g., denatonium benzoate, DB) is mixed into one vehicle infusate or a different intestinal taste cue is mixed into each vehicle infusate. *B*: mean licks/min for 0.12 M NaCl at the sipper spout across 30-min sessions in which rats received a yoked infusion during the first 6 min (shaded in gray) of either 10 mM DB mixed into LiCl, plain LiCl, or plain NaCl (replotted from Ref. 37). Thirsty rats consumed hypotonic NaCl at a high rate for an extended period of time when they were infused with either plain intraduodenal (ID) NaCl or plain ID LiCl, but beginning about 15 min after the infusion period, lick responses diverged. Rats slowed intake toward the end of the 30-min session following the plain LiCl. The unconditioned effects of LiCl infusions were evident in late session intake suppression (*LiCl malaise*). Rats receiving DB in the LiCl infusions rapidly suppressed ongoing intake by *minute 3* and rebounded intake 6 min later (*ID taste window*).

With respect to GI sweet taste signaling, this intestinal taste aversion paradigm provides a means to examine detection of early preabsorptive signals, apart from other postabsorptive effects of the nutritive stimulus. Thus the first aim of the present study was to determine whether rats are able to use intestinal nonnutritive sweet taste alone (Sucralose) to predict malaise. The finer temporal resolution offered by the current preparation may reveal the immediate effects of intestinal sweet taste on ongoing ingestion. However, in the event that sweet-tasting ID Sucralose is too weak on its own or responding extinguishes with repeated exposure, direct pairing with LiCl, a noncaloric, noncarbohydrate, but still biologically relevant stimulus, might maintain responding and augment detection over repeated trials. In our previous study (37), rats did not learn to use a different nonnutritive sweetener, 9.75 mM Na Saccharin, to predict LiCl, in this same paradigm; thus, here we used an artificial sweetener that more effectively stimulates cytosolic Ca^{2+} in oral sweet taste receptor cells and is a stronger stimulus for GLUT-2 upregulation in the intestine (25, 27). To complement this, the second aim of the present studies was to evaluate whether rats can use the early intestinal sensory properties of a functional carbohydrate, and arguably more conventional intestinal stimulus (sucrose), to predict malaise and then to discern whether the sweet taste feature of that cue was encoded. The latter was accomplished in a posttraining generalization test to intestinal nonnutritive sweet taste alone (Sucralose). If postoral sweet taste signaling is functionally linked to ingestive behavior, then it is predicted that rats should learn to respond to these immediate sensory effects of the training stimulus to rapidly suppress ongoing intake, and this response should readily generalize to other sweet-tasting compounds delivered to the duodenum.

GENERAL METHODS

Subjects

Male Sprague-Dawley rats (Harlan, Indianapolis, IN), approximately 90 days old at the start of the experiment, were individually housed in hanging wire mesh cages in a climate-controlled facility approved by the Association for the Assessment and Accreditation of Laboratory Animal Care. Lights were on at 06:00 and off at 18:00 (12:12 h light: dark schedule). Experiments were conducted in the light phase. Rats were fed ad libitum powdered chow (Purina no. 5001) and tap water during a 1-wk acclimation period and in a 2-wk postsurgery recovery period, before being switched to a restricted food and water access schedule for the remainder of the experiments (see *Deprivation schedule).* All animal care, surgical, and experimental procedures were approved by the Purdue University Animal Care and Use Committee. Efforts were made to minimize the number of animals used in the experiments and their discomfort.

Surgery

Each rat was fitted with an intraduodenal catheter according to procedures described in more detail elsewhere (37). Briefly, after an overnight fast, each rat was anesthetized (Nembutal, 60 mg/kg ip) and laparotomized. A Silastic catheter (ID = 0.64 mm, OD = 1.19 mm, Dow Corning, Midland, MI) was advanced through a puncture wound in the forestomach and then through the pyloric sphincter to where it was tethered to the duodenal wall (4 cm distal to the pyloric sphincter). The free end of the catheter was tunneled subcutaneously to an interscapular exit site and then exteriorized for connection to a

Luer-Lok adapter, which was mounted in a harness worn by the rat (Quick Connect Harness, Strategic Applications). Beginning 48 h after surgery, the catheter was flushed each day with 0.5 ml isotonic saline to maintain patency.

Apparatus

For 30 min each day, each rat had access to a controlled access lickometer (Habitest, Coulbourn Instruments, described previously in Ref. 37). Each lick at the sipper spout was recorded and operated a pump to deliver 5 μl of 0.12 M NaCl solution to the sipper spout (Graphic State, version 3.03). This 0.12 M NaCl was the only solution delivered to the oral sipper spout for consumption on each daily session. After a 6-day pretraining phase to familiarize rats with drinking NaCl at the sipper spout, a brief ID infusion was yoked to active licking in the first 6 min of each 30-min session. That is, during the first 6 min of each session, each lick at the sipper spout also operated a second pump for direct delivery of an infusate (5 μl/lick) to the duodenum. After this 6-min period elapsed, the ID infusion pump was rendered inactive, but rats were permitted to continue to lick for NaCl at the sipper spout for the remainder of the session. Before each session, the ID infusion lines were thoroughly flushed with 0.12 M NaCl to rinse out any remnants of the previous session's infusate from the line. The dead space in the line was filled with 0.12 M NaCl (~0.40 ml) before the line was attached to the syringe containing the ID stimulus. Then the rat was connected to the infusion line. At the end of the session, the rat's ID catheter was flushed with 2 ml of isotonic saline and capped.

Stimuli and Drugs

Properties of the probe training and testing stimuli are shown in Table 1. On all training and test sessions, the rats licked for 0.12 M NaCl, while being infused with NaCl or LiCl. LiCl was mixed to achieve the approximate final dosage of 8 mg per rat per ID LiCl session, while matching the molar concentration of the NaCl. Since rats self-infused ~4.6 ml of ID NaCl in pretraining, 0.12 M LiCl (e.g., 170 mg LiCl in 33 ml deionized water) was added to 0.12 M NaCl (e.g., 460 mg NaCl in 67 ml deinonized water) for a final LiCl concentration of 1.7 mg/ml. It is important to point out that a low dose of LiCl (about one-fifth of the dose used in conventional taste aversion experiments, see Ref. 29) was used here to keep intake stable across sessions and to avoid a situation in which the rats refused to drink, following a single exposure to LiCl. Intestinal taste cues were added to either the ID NaCl or LiCl, depending on training group assignment and test conditions (see *Experiments*). All solutions were prepared fresh each day with deionized water and delivered at room temperature. Reagents were purchased from Sigma Aldrich (St. Louis, MO).

Training and Testing Schedules

Deprivation schedule. After recovery from surgery (~2 wk), rats were gradually shifted onto a water and food deprivation schedule, in which they received one 30-min drinking session per day. Approximately 30 min after the conclusion of that drinking session, rats were given access to powdered chow and a 10-ml deionized water supplement for 5 h. After the initial acclimation to the schedule and for the remainder of the experiment, each rat was transferred to a lickometer

Table 1. *Properties of the oral solution and intestinal infusates for probe training and testing*

		pH	Milliosmole
All Experiments	*Oral Solution*		
NaCl	0.12 M	6.05	219
	Intestinal Infusates		
Experiment 1			
Suc in LiCl	234 mM Suc in 33% 0.12 M LiCl +67% 0.12 M NaCl	6.88	459
Suc in NaCl	234 mM Suc in 0.12 M NaCl	6.51	460
Sucra in LiCl	20 mM Sucra in 33% 0.12 M LiCl +67% 0.12 M NaCl	6.89	238
Sucra in NaCl	20 mM Sucra in 0.12 M NaCl	6.30	236
DB in LiCl	1 mM DB in 33% 0.12 M LiCl +67% 0.12 M NaCl	6.18	218
DB in NaCl	1 mM DB in 0.12 M NaCl	6.25	223
Experiment 2			
Sucra + Poly in LiCl	20 mM Sucra +80 mM Poly in 33% 0.12 M LiCl +67% 0.12 NaCl	4.54	327
Sucra + Poly in NaCl	20 mM Sucra +80 mM Poly in 0.12 M NaCl	4.56	340
DB in LiCl	1 mM DB in 33% 0.12 M LiCl +67% 0.12 M NaCl	6.18	218
DB in NaCl	1 mM DB in 0.12 M NaCl	6.25	223
Experiment 3			
Poly	80 mM Poly in 0.12 M NaCl	4.61	324
ACAR Poly	80 mM Poly in 0.12 M NaCl	6.15	333
PHL Poly	80 mM Poly +0.39% PHL in 0.12 M NaCl	4.55	330
Suc	234 mM Suc in 0.12 M NaCl	6.51	460
ACAR Suc	234 mM Suc +0.2% ACAR in 0.12 M NaCl	6.58	480
PHL Suc	234 mM Suc +0.39% PHL in 0.12 M NaCl	4.93	474
Sucra	20 mM Sucra in 0.12 M NaCl	6.30	236
ACAR Sucra	20 mM Sucra +0.2% ACAR in 0.12 M NaCl	6.56	248
PHL Sucra	20 mM Sucra +0.39% PHL in 0.12 M NaCl	5.42	227
NaCl	0.12 M	6.05	219
ACAR NaCl	0.2% ACAR in 0.12 M NaCl	6.54	237
PHL NaCl	0.39% PHL in 0.12 M NaCl	5.32	215
Gluc	444 mM Gluc in 0.12 M NaCl	6.08	686
ACAR Gluc	444 mM Gluc +0.2% ACAR in 0.12 M NaCl	6.56	706

NaCl, sodium chloride; DB, denatonium benzoate; Sucra, Sucralose; Suc, sucrose; Poly, Polycose; Gluc, glucose; ACAR, Acarbose; PHL, Phlorizdin.

chamber for the daily 30-min drinking session and then back to the homecage for the chow and water supplement.

Pretraining. Rats were pretrained to drink at the sipper spouts for 0.12 M NaCl for 30 min for 6 days (no ID infusions were made during these initial sessions). This was followed by 8 days in which rats were allowed to drink from the spouts for NaCl (for 30 min) and ID infusions of 0.12 M NaCl were yoked to licking in the first 6 min only of each session to establish extensive oral and intestinal experience with the safe salt solution. At the end of pretraining, 2 day average (last 2 days of pretraining) body weight, lick rate, total licks in the first 6 min, and total licks over the entire 30 min were calculated for each rat; then rats were assigned to probe training groups matched on the means ± SD of each of these four factors.

Probe training. In the probe training phase, rats received 10 (*experiment 1*) or 4 (*experiment 2*) 2-day trials. Each trial consisted of a pair of probe sessions, one session in which the 6-min ID infusate vehicle was 0.12 M NaCl and another in which the 6-min ID infusate vehicle was LiCl. These two probe sessions were run on consecutive days. The order of the probe session type within each 2-day trial was randomized across training (e.g., AB BA BA AB). Specific intestinal taste cues were added into each infusate type for training (see *Experiments* for descriptions of pairings). As with pretraining, rats always only consumed 0.12 M NaCl at the sipper spouts; thus probe sessions only differed on the basis of the type of infusate delivered to the intestine during the first 6 min of the session. One to three baseline restabilization sessions, in which rats licked for NaCl and were infused with NaCl vehicle (no tastants added), were administered between each two-session probe trial.

Testing. After the probe training phase in *experiments 1* and *2*, generalization tests were conducted. On all tests, as with training, rats licked at the sipper spouts for plain 0.12 M NaCl for 30 min, while receiving a brief (first 6 min) yoked ID infusion. In *experiment 1*, two test sessions were compared, one test in which 20 mM Sucralose in 0.12 M NaCl was the ID infusate and a second test in which 1 mM DB in 0.12 M NaCl was the ID infusate in counterbalanced order. In *experiment 2*, rats were given the same generalization tests, except that each test was conducted on the day after an ID LiCl (no tastant added) reinstatement (yoked to licking in the first 6 min) session.

In *experiment 3*, the early intake-suppressive effects of three ID infusates (20 mM Sucralose, 234 mM sucrose, and 80 mM Polycose) on ongoing 0.12 M NaCl intake were examined in separate test sessions, in which each ID infusate was administered as is in 0.12 M NaCl (no-drug control) or along with a blocker-Acarbose or Phloridzin. These nine tests were presented in a randomized order for each rat, with the rule that no two same drug treatments were presented on sequential sessions. Acarbose (0.2%, ACAR, Roxane Laboratories) was made by first dissolving two 50-mg tablets into 50 ml of deionized water. Then the inactive ingredients (e.g., magnesium stearate, talc) were removed by centrifugation at 2,000 rpm for 10 min (Beckman Coulter Allegra x-15R). Phloridzin (0.39%, PHL, Sigma) was prepared in deionized water. NaCl was added to each drug solution to make it 0.12 M. Then intestinal tastants were added to these blocker solutions for ID delivery at test.

Statistical Analyses

All analyses were conducted with Statistica (version 10, Statsoft, Tulsa, OK), and data were graphed with Graphpad Prism (version 5.04, La Jolla, CA). Probe training data were averaged into blocks of two trials for statistical analyses. For *experiments 1* and *2*, a training group by probe infusate by trial block by minute repeated measures ANOVA was initially conducted. For *experiment 3*, separate drug by minute repeated measures ANOVAs were run for each test infusate after an initial ANOVA comparing the test infusates under the no drug control condition. In all cases where appropriate, post hoc ANOVAs or Newman-Keuls tests were run to break down significant main effects and interactions from the overall analyses. Finally, to examine early changes in intake in response to the intestinal taste properties of the infusate stimulus, additional analyses were conducted on the *taste window*, defined as the 6-min period following the average highest point of intake (e.g., *minutes 5–10* in *experiment 1*, see Fig. 1). Lick rate and/or intake suppression during the taste window (as the difference in lick rate on one probe session from the alternative probe session) was calculated and analyzed between training groups, across trials, or drug treatment conditions with ANOVA. An α level of 0.05 was used for all analyses.

RESULTS

Experiment 1

Experiment 1 examined the efficacy of two sweet stimuli in an intestinal taste aversion. One cue (Sucralose, Sucra), an artificial sweetener, has only preabsorptive sensory effects in the intestine (42), whereas the second cue (sucrose, Suc) comprises sweet taste alongside other GI and postabsorptive effects. Four groups of rats were compared here. In this factorial discrimination design, all rats received one sweet cue and one bitter cue consistently laced into either ID NaCl or ID LiCl for training. For half of the rats, the sweet cue was added to the ID LiCl and the bitter cue to the ID NaCl, whereas the other half received the reverse contingency (bitter LiCl, sweet NaCl). For half of the rats, the sweet cue was 20 mM Sucra and for the other half the sweet cue was 234 mM Suc. This low concentration of Suc was chosen to minimize the negative feedback from calories and/or osmolality during the 30-min drinking session. In our previous study (37), there was some concern that rats did not respond to Na Saccharin as a probe stimulus because it was overshadowed by the salient stimulus presented on alternative sessions (10 mM DB). Therefore, in the present experiment, while the taste cue mixed into the alternative probe vehicle was still bitter DB to enhance discrimination, it was presented at a much lower concentration (1 mM) to avoid such cue competition. This additionally permitted us to assess whether a much weaker DB stimulus paired with LiCl was detected in the intestine to control ingestion. After training, rats were given a two-session generalization test: one session in which the ID stimulus was 20 mM Sucra in NaCl and a second session in which the ID stimulus was 1 mM DB in NaCl. It was expected that if rats trained with ID Suc in LiCl learned about its sweet taste properties, responding should subsequently generalize to the sweet taste of ID Sucra alone.

Probe training. Figure 2 presents the mean lick rates across the ID NaCl and LiCl probe infusate sessions as a function of training group. Overall, ANOVA yielded a significant main effect of probe infusate $F(1, 22) = 20.16$, $P = 0.0002$, and significant training group by probe infusate $F(3, 22) = 3.17$, $P = 0.04$, and training group by probe infusate by minute interactions $F(87, 638) = 1.40$, $P = 0.01$. Separate follow-up analyses were conducted for each training group.

The *Suc in LiCl*-trained group (Fig. 2*A*) consumed significantly less on sessions in which they were infused with that Suc in LiCl probe (125.98 ± 19.17 licks/min) compared with the alternative DB in NaCl probe (157.59 ± 22.16 licks/min) ($P = 0.003$). Collapsed across training, these rats began to suppress licking in response to the Suc in LiCl infusate early in the session (*minutes 7* and *9*, $P = 0.08$), $F(29, 203) = 1.75$, $P = 0.01$. The relative early lick suppression to Suc in LiCl emerged across trial blocks $F(116, 812) = 1.42$, $P = 0.004$. On *trial block 1*, the *Suc in LiCl* group did not discriminate the

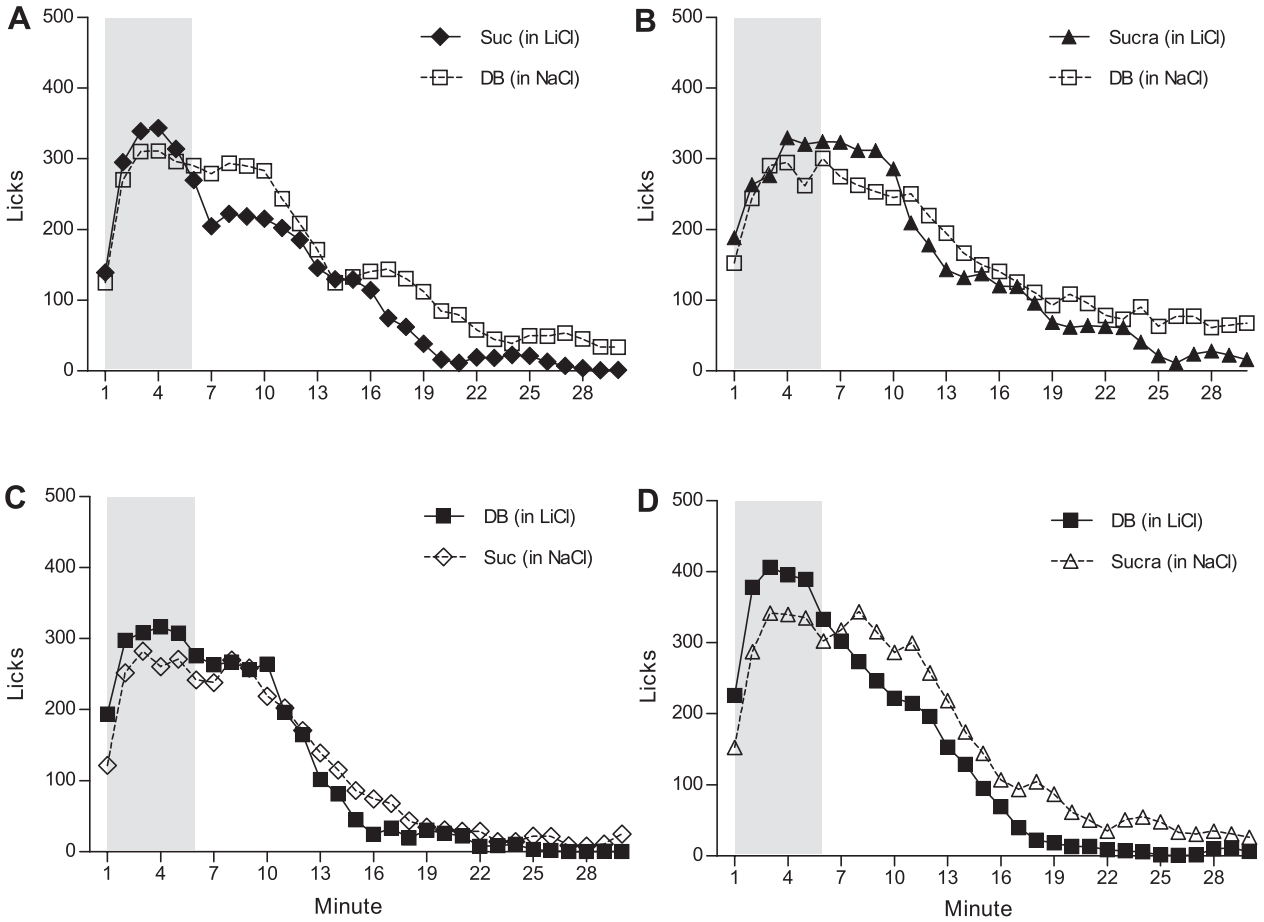

Fig. 2. Mean licks/min for a 0.12 M NaCl solution at the sipper spout collapsed across ten 30-min probe trials. A brief (6 min) ID infusion was yoked to licking at the beginning of the 30-min session (shaded area). *A*: *Suc in LiCl* group ($n = 8$) received 234 mM Sucrose (Suc) mixed into LiCl infusions on one session per trial and received 1 mM DB mixed into NaCl infusions on the alternative session per trial. *B*: *Sucra in LiCl* group ($n = 7$) received 20 mM Sucralose (Sucra) mixed into LiCl infusions on one session per trial and 1 mM DB mixed into NaCl infusions on the alternative session per trial. *C*: *Suc in NaCl* group ($n = 5$) received 1 mM DB mixed into LiCl on one probe session and 234 mM Suc mixed into NaCl on the alternative probe session per trial. *D*: *Sucra in NaCl* group ($n = 6$) received 1 mM DB in LiCl on one probe session and 20 mM Sucra in NaCl on the alternative probe session per trial.

early sensory properties of the two ID probe infusates, but intake was suppressed late in the session following LiCl infusions (during the *LiCl malaise* window) ($P = 0.04$). By *trial block 2*, intake began to decay slightly earlier on the Suc in LiCl session (at *minute 11*, $P = 0.02$) compared with the DB in NaCl session. Intake suppression in response to the ID Suc in LiCl occurred earlier and earlier across the next two trial blocks. By the fifth and final trial block, rats significantly suppressed intake by *minutes 7–8* ($P = 0.01$ and 0.009, respectively) and again at *minute 17* [$P = 0.02$, $F(29, 203) = 2.55$, $P = 0.000001$].

Rats that received the opposite training contingencies *Suc in NaCl* (Fig. 2*C*) consumed similar amounts on both probe sessions (Suc: 118.60 ± 28.03, DB: 117.56 ± 24.25 licks/min, $P = 0.89$). In fact, lick patterns across both of these session types corresponded near perfectly with one another, with the exception that rats consumed more on *minute 1* of DB in LiCl sessions [($P = 0.05$), $F(29, 116) = 2.11$, $P = 0.002$]. Even the late session intake suppression following the Suc in NaCl infusion closely resembled that following the DB in LiCl infusion; this may reflect an unconditioned satiating effect of the Suc that arises with about the same latency as the unconditioned effects of LiCl. Intake decreased across training [$F(4, 16) = 4.01$, $P = 0.02$], but this was irrespective of probe infusate type [$F(4, 16) = 1.20$, $P = 0.35$]. No significant lick pattern divergences at any point across the session emerged with training [$F(116, 464) = 1.09$, $P = 0.28$]. Because these analyses that compared responding to ID Sucra and ID DB failed to yield any indication that rats learned to predict malaise with this low concentration (1 mM) ID DB, a separate analysis was conducted to look at the response to just the DB in LiCl probes across trial blocks. This further confirmed that latency to suppress licking remained consistent from *trial block 1* to *trial block 5*, with rats generally slowing intake *minutes 10–12* of the DB in LiCl sessions [$F(116, 464) = 1.20$, $P = 0.10$].

Overall, the *Sucra in LiCl* group (Fig. 2*B*) consumed only slightly (and nonsignificantly) less on their ID Sucra sessions (151.88 ± 20.50 licks/min) compared with the their ID DB sessions (162.99 ± 23.69 licks/min) ($P = 0.34$); this small difference was due to a termination in ongoing ingestion in the concluding minutes of the Sucra in LiCl sessions [malaise window, closest at *minute 26*, $P = 0.09$, $F(29, 174) = 3.62$, $P = 0.00001$]. There was no indication that rats responded to the early intestinal cue properties of Sucra in anticipation of the LiCl malaise. Furthermore, despite a near-significant trial block by probe infusate by minute interaction [$F(116, 696) =$

1.23, $P = 0.07$] separate analyses on each trial block failed to yield a systematic pattern, suggesting that ID Sucra in LiCl did not gain control over early ingestion, even after several exposures to the training contingencies. Intake did, however, decay more rapidly toward the end of the 30-min sessions (malaise window), following Sucra in LiCl infusions on *trial block 4* ($P = 0.01$). By the fifth and final trial block, *Sucra in LiCl*-trained rats consumed marginally less on the ID Sucra session compared with the ID DB session [$F(1, 6) = 5.38$, $P = 0.06$], but again, if anything, this was due to a difference in the concluding minutes of the session. Moreover, although total intake on the Sucra in LiCl session decreased following the first trial block [$F(4, 24) = 2.99$, $P = 0.04$], the pattern of licking across the entire 30 min, but especially around the infusion period, did not significantly change across probe training [$F(116, 696) = 0.82$, $P = 0.91$].

The *Sucra in NaCl* group (Fig. 2*D*) consumed significantly less on their DB in LiCl probe sessions (139.54 ± 22.14 licks/min) compared with their Sucra in NaCl probe sessions (165.52 ± 25.59 licks/min) ($P = 0.02$). This was primarily due to an earlier reduction in licking on DB in LiCl sessions (beginning at *minute 11*, $P = 0.03$) [$F(29, 145) = 3.53$, $P = 0.000001$]. Overall, this pattern did not significantly change across training [$F(116, 580) = 1.15$, $P = 0.16$]. But it is important to note that, as with the other training groups, the *Sucra in NaCl* group did not discriminate among the two probe infusates on the first trial block [$F(29, 145) = 0.81$, $P = 0.74$]. As early as *trial block 2*, the response to DB in LiCl emerged [$F(29, 145) = 1.78$, $P = 0.01$] and was maintained for the remainder of training (intake suppression beginning at *minute 8* on *trial block 4* and *minute 10* on *trial block 5*, $P = 0.006$ and 0.001, respectively) [significant probe infusate by minute interactions, $F(29, 145) = 2.43$, $P = 0.0003$ and $F(F(29, 145) = 2.79$, $P = 0.00003$].

Figure 3*A* presents lick rate suppression to early properties of the intestinal cue paired with LiCl from the alternative cue paired with NaCl during the taste window (*minutes 5–10*) for each training group. All training groups suppressed intake across trial blocks [$F(4, 88) = 6.26$, $P = 0.0002$]. However, only for the *Suc in LiCl* group was this due to a significant reduction in licking on the LiCl session from the NaCl session across the taste window period [significant at *minutes 7–10*, largest $P = 0.007$ at *minute 10*) $F(5, 35) = 4.48$, $P = 0.003$; this differential responding strengthened across training (from trial block 1 to trial block 5, $P = 0.02$]. The *Sucra in NaCl* group tended to consume less on the DB in LiCl session during the taste window, but this effect was only marginally significant [$F(1, 5) = 5.01$, $P = 0.08$]. For these rats, intake on the DB in LiCl sessions diverged late in the taste window period [*minutes 8–10*, largest $P = 0.051$ at *minute 8*), $F(2, 25) = 2.69$, $P = 0.04$]; however, these patterns are most likely accounted for by a spurious robust difference at *trial block 4* only [$F(4, 20) = 2.91$, $P = 0.05$]. Both the *Sucra in LiCl* and *Suc in NaCl* groups failed to significantly reduce intake on their LiCl session from their NaCl session during the taste window ($P = 0.79$ and 0.78, respectively) at any point across training ($P = 0.11$ and 0.66, respectively).

Testing. Three rats were dropped from the experiment during this test phase due to catheter failure. Unfortunately, these rats all belonged to the *Suc in NaCl* training group, leaving an insufficient *n* for statistical comparisons; therefore, the remaining *Suc in NaCl*-trained rats were excluded from all statistical analyses. Overall, rats responded to the two test infusates in a similar manner across the 30-min tests. This was indicated by a nonsignificant test by minute interaction [$F(29, 435) = 0.78$, $P = 0.78$], which also did not further interact with training group [$F(58, 435) = 1.15$, $P = 0.23$]. However, closer examination of the early taste window (*minutes 5–10*, Fig. 3*B*) revealed that rats trained with *Suc in LiCl* generalized that learned response to ID Sucra alone at test by suppressing intake in response to the Sucra infusion, relative to the DB infusion during the taste window. This suppression was marginally greater than that observed for rats trained with *Sucra in LiCl* [$F(1, 10) = 3.50$, $P = 0.09$] and *Sucra in NaCl* [$F(1, 9) = 3.07$, $P = 0.11$]. In fact, both the *Sucra in LiCl* and *Sucra in NaCl* groups failed to differentially respond to the two test infusates during the taste window (closest $P = 0.75$ at *minute 9* and $P = 0.81$ at *minute 8*) or at any point across the 30-min session

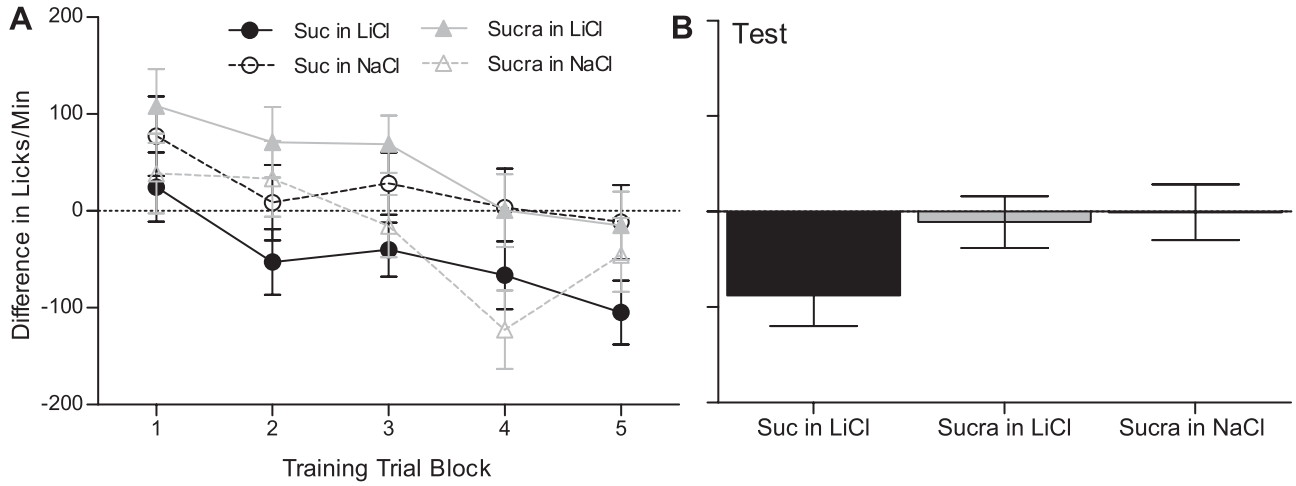

Fig. 3. *A*: means ± SE suppression in licks/min for 0.12 M NaCl at the sipper spout on the ID LiCl session from the ID NaCl session during the intestinal taste window (*minutes 5–10*) as a function of trial block and training group. *B*: means ± SE suppression in licks/min for 0.12 M NaCl at the sipper spout on the 20 mM Sucra alone in NaCl test from the 1 mM DB in NaCl test during the intestinal taste window (*minutes 5–10*) for each group (*Suc in LiCl, n* = 5; *Sucra in LiCl*, *n* = 7; *Sucra in NaCl*, *n* = 6).

(closest $P = 0.44$ at *minute 22* and $P = 0.56$ at *minute 22*, respectively).

Discussion. The results showed that rats trained with *Suc in LiCl* developed an early and transient lick rate suppression in response to the arrival of the Suc in the intestine, after several exposures to the training contingencies. In contrast, those that received *Sucra in LiCl* failed to develop an early lick rate suppression, even after 10 exposures to the stimulus pairings. As such, these results could be taken to suggest that rats do not learn about sweet taste per se, but instead learn about some other stimulus feature of the Suc in the intestine. However, the posttraining test indicated that *Suc in LiCl*-trained rats rapidly responded to the arrival of ID Sucra. This generalization pattern suggests that the sweet taste property of Suc was encoded at training.

The differences in efficacy of Suc and Sucra to associate with LiCl may be due to differences in stimulus intensity. Certainly, Suc has different postingestive effects, both preabsorptively and postabsorptively, than Sucra. In taste learning as with other modalities, the strength and rate of conditioning is positively associated with the intensity of the conditioned and unconditioned stimuli (9, 29). That said, if it were simply the case that ID Sucra was a weaker stimulus, then response acquisition would expected to be retarded but not entirely lacking. Thus the fact that there were no indications of a response developing to Sucra with such extensive training seems more indicative of insensitivity to the stimulus.

Interestingly, however, rats in the *Suc in LiCl* group responded rather robustly to Sucra during the posttraining generalization test, suggesting their training history with Suc enhanced responding to the same stimulus that the *Sucra in LiCl* group did not appear to respond to despite, in that case, extensive pairing with LiCl. Potentiation appears to be especially important to taste aversions, as it is a means of conferring information about a target stimulus onto an associated antecedent stimulus to detect the potential toxin as early as possible (14). Perhaps then, the associative processing of the compound stimulus properties of ID Suc or the more direct influence of those corollary signals at nearby preabsorptive receptors or on a postabsorptive feedback mechanism, potentiated responding to the sweet taste properties of Suc (see also GENERAL DISCUSSION).

Experiment 2

To further assess whether intestinal sweet taste detection is potentiated by being presented in compound with a functional carbohydrate stimulus during training, rats previously trained with *Sucra in LiCl* (on alternating probe sessions with DB in NaCl) were given additional aversion training with Sucra in compound with 80 mM Polycose (Poly) in LiCl and 1 mM DB in NaCl on alternate probe sessions for four trials. This group was compared with those rats previously trained with *Sucra in NaCl* (on alternating probe sessions with DB in LiCl), who now received Sucra+Poly in NaCl on alternate probe sessions as DB in LiCl for four trials. It was predicted that rats now trained with Sucra+Poly in LiCl would develop an earlier suppression in intake to that stimulus in training, compared with a group that received Sucra+Poly in NaCl. After this training, both groups of rats were tested for their responding to the ID Sucra component alone (in NaCl), relative to ID DB (in NaCl). Because of the limited number of training trials, tests were conducted in reinstatement to maintain responding to the cue features across testing. Thus, on the day before each test, plain LiCl was the ID infusate to provide a malaise reminder. It was predicted that only the *Sucra+Poly in LiCl* group would respond to the ID Sucra component alone at test.

Probe training. Figure 4 presents mean licks/min across the Sucra+Poly and DB probe sessions collapsed across four trials for each training group. Both groups consumed significantly less on their ID Sucra+Poly sessions (*Sucra+Poly in LiCl* group: 105.86 ± 21.06 licks/min; *Sucra+Poly in NaCl* group: 143.58 ± 28.25 licks/min) compared with their ID DB sessions (*Sucra+Poly in LiCl* group: 210.98 ± 30.95 licks/min, $P = 0.01$; *Sucra+Poly in NaCl* group: 208.22 ± 19.23 licks/min, $P = 0.04$), but the difference was more pronounced in the group for which Sucra+Poly was paired with LiCl [$F(1, 9) = 21.21$, $P = 0.001$]. This difference among groups was primarily due to an earlier and more robust lick suppression following the Sucra+Poly probe in the *Sucra+Poly in LiCl* group [significant training group by probe infusate by minute interaction, $F(29, 261) = 4.73$, $P = 0.000001$]. Post hoc analyses con-

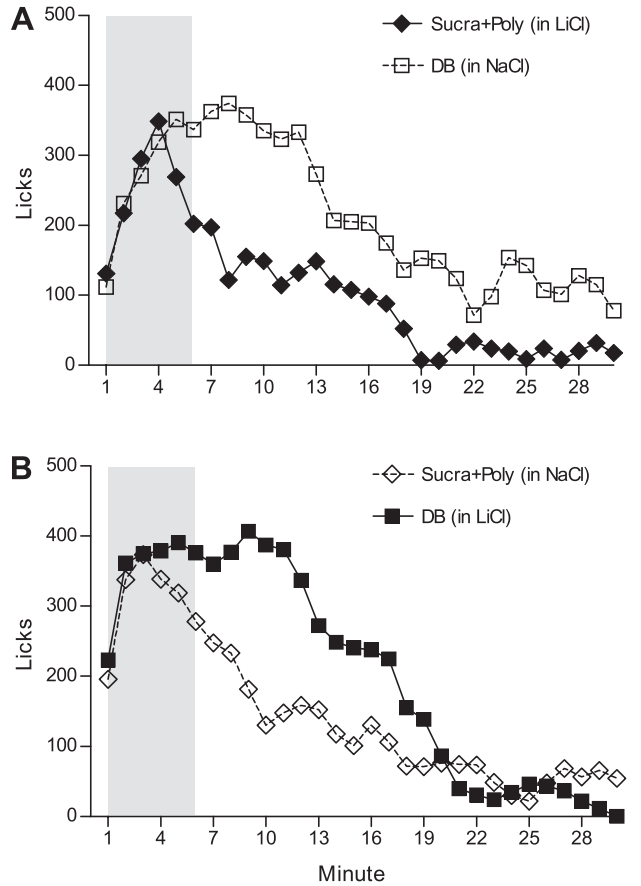

Fig. 4. Mean licks/min for 0.12 M NaCl at the sipper spout collapsed across four 30-min probe trials. A brief (6 min) ID infusion was yoked to licking at the beginning of the 30-min session (shaded area). *A*: rats previously trained with *Sucra in LiCl* ($n = 5$) received 20 mM Sucra and 80 mM Poly mixed into LiCl infusions on one session in each trial and 1 mM DB mixed into NaCl infusions on the alternative session in each trial. *B*: rats previously trained with *Sucra in NaCl* ($n = 6$) received 20 mM Sucra and 80 mM Poly mixed into NaCl infusions on one session in each trial and 1 mM DB mixed into LiCl on the alternative session in each trial.

firmed that the *Sucra+Poly in LiCl* group suppressed licking 1 min earlier in response to the Sucra+Poly probe compared with the DB probe [*minutes 8–12*, largest $P = 0.04$ at *minute 10*) $F(29, 116) = 1.85$, $P = 0.01$] than did the *Sucra+Poly in NaCl* group [*minute 9–12*, largest $P = 0.002$ at *minute 12*), $F(29, 145) = 4.40$, $P = 0.000001$]. This difference was not evident on the first trial block but emerged on the second trial block.

ANOVA conducted to compare responding on the Sucra+Poly probe and DB probe across trial blocks between training groups returned a significant trial block by group by probe infusate by minute interaction [$F(29, 261) = 1.56$, $P = 0.04$]. Separate analysis on the *Sucra+Poly in LiCl* group indicated that these rats more rapidly and robustly suppressed licking in response to the Sucra+Poly probe on *trial block 2* than on *trial block 1* [$F(29. 116) = 1.51$, $P = 0.07$]. Specifically, in the first trial block, the *Sucra+Poly in LiCl* group consumed less in the Sucra+Poly sessions than in the DB sessions [$F(1, 4) = 23.32$, $P = 0.008$]. This was due to a suppression in lick rate on the Sucra+Poly sessions from the alternative DB sessions, beginning around *minute 8*, but the infusate by minute interaction did not achieve significance [$F(29, 116) = 1.01$, $P = 0.47$]. In fact, on this first trial block, the *Sucra+Poly in NaCl* group evinced comparable responses to each of the ID probe tastants as the *Sucra+Poly in LiCl* group [$F(29, 261) = 1.06$, $P = 0.39$]. Differences between the training groups emerged on the second trial block [$F(29, 261) = 1.17$, $P = 0.02$]. Whereas the *Sucra+Poly in LiCl* group exhibited a robust early lick suppression in response to Sucra+Poly (*minutes 7–13*, largest $P = 0.02$ and 0.06 for *minutes 9* and *10*, respectively), relative to the alternative DB probe [$F(29, 116) = 2.63$, $P = 0.00001$], the *Sucra+Poly in NaCl* group maintained a consistent intake pattern to each of the probes on the second trial block from the first trial block [$F(29, 145) = 1.13$, $P = 0.31$], significantly reducing intake on the Sucra+Poly sessions at *minute 9* [($P = 0.04$), $F(29, 145) = 2.58$, $P = 0.0001$].

Figure 5 presents the mean change in early lick rate suppression (the difference between lick rate Sucra+Poly probe and the DB probe) during the taste window (*minutes 4–9*) across the two trial blocks. ANOVA yielded a significant training group by trial block by minute interaction [$F(5, 45) = 2.87$, $P = 0.02$]. Post hoc tests on the Sucra+Poly probe sessions confirmed that both groups licked at comparable rates across the taste window on *trial block 1* [$F(5, 45) = 0.55$, $P = 0.74$]. On *trial block 2*, however, the *Sucra+Poly in LiCl* group exhibited a more rapid and robust reduction in intake in response to the Sucra+Poly probe, especially in the latter half of the taste window [$F(5, 45) = 2.39$, $P = 0.053$]. Early responding to the alternative DB probe remained consistent from *trial block 1* to *trial block 2* for both training groups [closest for the training group by trial block by minute, $F(5, 45) = 1.09$, $P = 0.38$].

Testing. Figure 5 presents the mean difference in lick rate during the taste window (*minutes 4–9*) of the ID Sucra alone test from the ID DB alone test for each of the training groups. The *Sucra+Poly in LiCl* group rapidly suppressed intake by *minute 5* ($P = 0.03$) of the Sucra alone test and then returned to DB test intake levels shortly thereafter [$F(5, 10) = 3.61$, $P = 0.04$]. By contrast, the *Sucra+Poly in NaCl* group did not respond differentially to the two test infusates during the taste

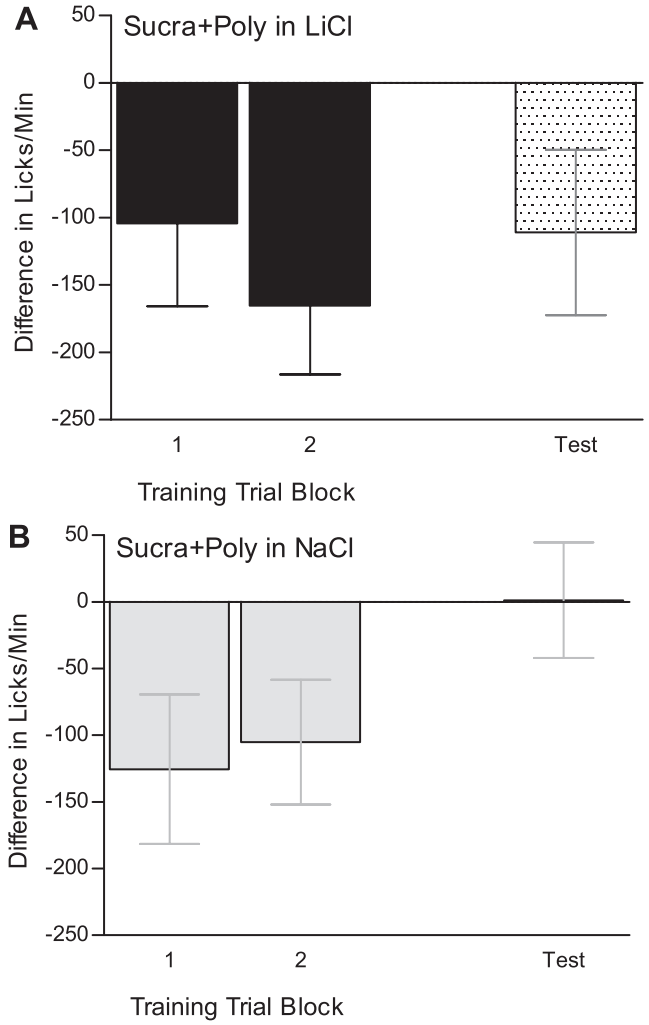

Fig. 5. *A*: means ± SE suppression in licks/min for 0.12 M NaCl at the sipper spout during the taste window (*minutes 4–9*) in response to the ID Sucra+Poly probe (difference from the ID DB probe) across training ($n = 5$) and licks/min suppression in response to the ID Sucra component alone probe (difference from the ID DB probe) at test ($n = 3$) for the *Sucra+Poly in LiCl* group. *B*: means ± SE suppression in licks/min for 0.12 M NaCl at the sipper spout during the taste window (*minutes 4–9*) in response to the ID Sucra+Poly probe (difference from the ID DB probe) across training and licks/min suppression in response to the ID Sucra component alone probe (difference from the ID DB probe) at test for the *Sucra+Poly in NaCl* group ($n = 6$). Tests were conducted in reinstatement.

window [closest for the test by minute interaction, $F(5, 25) = 0.17$, $P = 0.97$] or any point across the 30-min test sessions [$F(29, 145) = 0.50$, $P = 0.98$].

Discussion. The results showed that rats that previously failed to respond to Sucra in LiCl with extensive training, quickly developed an early lick rate suppression to the same sweet stimulus now presented in compound with Poly for training. In a pattern consistent with *experiment 1*, *Sucra+Poly in LiCl*-trained rats subsequently responded to the nonnutritive intestinal sweet taste cue (Sucra) alone at test. Without additional experimental groups (particularly a group that did not have extensive training history with Sucra in LiCl), this outcome might be taken to suggest only that additional training with the nonnutritive sweetener is necessary to establish responding to ID Sucra. However, the sudden and rapid acqui-

sition of a response to Sucra with this second training phase with Poly certainly argues that some interaction between Sucra and Poly greatly enhances responding to intestinal sweet taste alone.

Bitter DB (1 mM) delivered at a much lower concentration than in our previous study (10 mM, Ref. 37) did not prove to be a salient intestinal stimulus on its own (in NaCl), and direct pairing with LiCl failed to substantially augment detection, even after 10 trials (*experiment 1*) and four additional trials (*experiment 2*). The effect of 1 mM DB in LiCl was most obvious toward the end of training for the *Sucra in NaCl* group (*experiment 1*), but, even in this case, rats did not respond to DB when it was transferred to an ID NaCl vehicle at test, suggesting the training effect may have instead reflected an unconditioned effect of the LiCl or responding was so weak that the effect rapidly extinguished during testing. Moreover, additional ID DB in LiCl training for this group in *experiment 2* did not yield indications that these rats were responding to the early cue properties of 1 mM DB in the intestine. A previous study in 2008 by Glendinning et al. (15) found that intragastric infusions of 2.5 mM DB failed to reinforce flavor avoidance, suggesting such low concentrations are not inherently aversive at the level of the postoral GI tract. The results of the present study suggest that these near-oral threshold DB concentrations (45) may be subthreshold for the postoral receptors.

Experiment 3

Experiments 1 and *2* suggest that rats may be relatively insensitive to the sweet taste of Sucra in the duodenum, without prior training with either a sweet-tasting carbohydrate or sweet taste in combination with a second carbohydrates. One possibility is that the corollary effects of carbohydrate effectively sensitize the sweet taste signal. These corollary effects may even occur through an alternative preabsorptive receptor. In addition to sweet taste receptors, the GI tract uses sodium-glucose-linked tranporters (SGLTs) to signal the presence of glucose in the lumen (8). SGLTs are perhaps akin to the ion receptors (e.g., salt, Ref. 43) on the tongue insofar as the process of transporting molecules (in this case glucose and Na) into the enterocyte (or entero-endocrine cell) is also the event that depolarizes the cell (21). However, these transporters are highly specific to glucose and their effects appear to be sensory, insofar as nonmetabolizable glucose analogues likewise stimulate these transceptors (11, 24). Previous studies on glucose transport have suggested that initial activity at the SGLTs (but also GLUTs) depolarizes the cell and, in doing so, activates nearby T1Rs (for a review see Ref. 21).

Alternatively, it could be the case that Sucra is a relatively poor ligand for the intestinal sweet taste receptors, but Suc and Poly (i.e., hydrolyzed to glucose) sufficiently stimulate these sweet receptors in training. Oral sweet taste receptors have a high affinity for Sucra and sweet-tasting carbohydrates like Suc (30, 31, 56), but there is some reason to believe that the receptors in the GI tract may differ in their sensitivity and affinity for such ligands. In oral taste tissue, T1Rs are primarily functional only as heterodimers (T1R2+T1R3), though a few solitary T1R3s may function as low affinity sweet receptors as well (30, 55). But in the stomach and intestines, there is virtually no evidence to suggest that the various T1R subtypes are even expressed within the same cell (except see one example in Ref. 28). Moreover, on the tongue, T1R3s commonly use a PLCβ-2 signaling cascade, but, in the duodenum, T1R3s are rarely found to associate with this same signaling protein (3). These unique molecular profiles could very well underlie differences in ligand affinity and receptor sensitivity.

Given such questions about the compatibility of the signaling pathways and ligand affinities, the present experiment was designed to assess whether the sweet taste of Suc is sufficient to signal its early negative feedback on ongoing ingestion, separate from its effects through alternative signaling pathways. Rats previously exposed to ID Suc, Sucra, and/or Sucra+Poly paired with LiCl or NaCl (opposite DB) and then posttraining generalization testing, now received, on one set of tests, a test probe (Suc or Poly) mixed with Acarbose (ACAR). ACAR is a competitive mucosal α-glucosidase inhibitor, which prevents the hydrolysis of di- and polysaccharides into their monosaccharide units. On the one hand, this interferes with each compound's metabolic effects. But this treatment is of particular interest because intact Poly is considered a non-sweet-tasting carbohydrate, while intact Suc binds with sweet taste receptors and is considered sweet tasting (57). Thus, if ACAR interferes with intestinal Poly, but not Suc, detection, then it would be indicative of a functional preabsorptive sweet taste receptor, independent of SGLT/GLUT or other postingestive effects.

In a second set of tests, rats received the test probe (Suc or Poly) mixed with Phloridzin (PHL). PHL is a competitive SGLT inhibitor. If Poly detection is completely blocked by PHL treatment, then it follows that SGLT activity is a necessary component of the preabsorptive signaling pathway for GI glucose detection. On the other hand, if early ID Poly (now cleaved to glucose) detection remains intact under PHL treatment, then it follows that an alternative sweet receptor (e.g., T1R) is at the very least sufficient. Moreover, it was hypothesized that Suc detection may only be somewhat hampered with PHL treatment; this is because fructose transported through the uninhibited GLUT-5 may depolarize the cell to generate a signal on its own or additionally activate sweet taste pathways. Both treatments, ACAR and PHL, were compared with a no-drug control for each test infusate. Additionally, three control test infusates, Sucra, plain NaCl, and equicaloric glucose (Gluc), were used to rule out nonspecific effects of the drugs on intake.

Figure 6 presents the mean lick response during the taste window (*minutes 5–10*) of the three test infusates, under no-drug, ACAR, and PHL treatment conditions. Under the no-drug baseline condition, a main effect of test infusate [$F(2, 20) = 9.87$, $P = 0.001$], indicated that rats more rapidly and robustly reduced intake in response to the Poly infusion compared with the Sucra infusion ($P = 0.009$) and equicaloric Suc infusion ($P = 0.01$). Intake on the Suc test was somewhat reduced relative to intake on the Sucra test during the taste window ($P = 0.13$). Separate ANOVAs yielded a significant and near significant main effect of drug treatment for both the Poly and Suc tests [$F(2, 20) = 18.35$, $P = 0.00003$ and $F(2, 20) = 3.16$, $P = 0.06$]. Post hoc tests confirmed that ACAR treatment completely abolished the early detection of both ID Poly ($P = 0.0002$) and ID Suc ($P = 0.06$), but PHL, on the other hand, impaired the early detection of ID Poly ($P = 0.0002$) and had no effect on responding to ID Suc ($P = 0.76$).

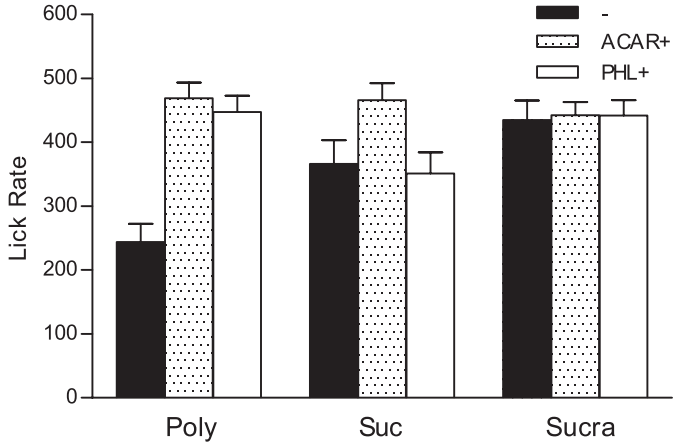

Fig. 6. Means ± SE licks/min for 0.12 M NaCl at the sipper spout during the taste window (*minutes 5–10*) on test sessions in which ID infusions of test solutions (80 mM Poly in NaCl, 234 mM Suc in NaCl, or 20 mM Sucra in NaCl) were yoked to the first 6 min of licking ($n = 11$). Test infusates were mixed in Acarbose (ACAR+), phloridzin (PHL+), or alone (no drug control, −).

ANOVAs failed to return significant main effects of drug treatment for the ID Sucra and ID NaCl tests [no-drug NaCl: 405.97 ± 25.51; ACAR NaCl: 430.39 ± 30.01; PHL NaCl 452.56 ± 27.52 licks/min), $F(2, 20) = 0.08$, $P = 0.93$ and $F(2, 20) = 2.25$, $P = 0.13$]. This suggests that neither ACAR nor PHL produced nonspecific effects on intake. Moreover, ACAR did not attenuate detection of the early sensory properties of the sweet monosaccharide Gluc [no drug: 235.27 ± 44.11; ACAR: 206.35 ± 48.33 licks/min), $F(1, 10) = 0.20$, $P = 0.66$].

Discussion. The purpose of *experiment 3* was to determine whether sweet taste alone, separate from SGLT activity, was sufficient to signal the presence of carbohydrate calories in the intestine and terminate ongoing ingestion, by preventing the hydrolysis of Suc and Poly with ACAR, and by blocking the SGLTs, the other putative glucose sensors, with PHL. Consistent with a previous study by Savastano et al. (36), which used a preload infusion and extended feeding test paradigm, ACAR completely abolished the early satiating effects of Poly. These results suggest that at the very least Poly must be hydrolyzed to glucose to be detected. Of particular interest with respect to the previous experiments in this paper, was whether the same was true for Suc, a disaccharide that is, by oral taste standards, a ligand for the T1Rs. Results clearly showed that ACAR likewise completely abolished the early intestinal detection of intact Suc. In fact, the comparable effects of ACAR on Suc and Poly detection go some way to ruling out the adequacy of sweet taste alone to signal the presence of these compounds in the intestine. The results of the PHL test further suggest that activity at the SGLTs is essential for early Poly (i.e., glucose) detection. The control tests with Sucra, NaCl, and glucose suggest that ACAR and PHL did not nonspecifically increase or decrease intake, respectively.

GENERAL DISCUSSION

The present studies demonstrated that rats were able to use intestinal carbohydrate cues to anticipate malaise and suspend ongoing intake. Posttraining tests indicated that the sweet taste property of Suc was encoded during training, such that rats responded to ID sweet taste (Sucra) alone at test. Under these conditions, in the absence of the other postingestive effects of Suc at test, rats suppressed intake within about 3 min of the start of the session and resumed normal intake shortly thereafter, closely resembling the tight stimulus-bound pattern observed for ID bitter DB (10 mM) in our previous experiment (37). Interestingly, even though conditioning to ID Suc appeared to transfer to ID Sucra at test, rats failed to exhibit evidence of learning about ID Sucra alone during aversion training. This is consistent with our previous finding with a different artificial sweetener, Na Saccharin (37). The present experiment went on to show, however, that presenting that artificial sweet taste in compound with a functional carbohydrate (Poly) augments detection and responding to the sweet taste component alone in the intestine. This was not likely due to some interaction between Sucra and Poly at the intestinal sweet taste receptors but rather due to some influence of Poly's activity at the SGLTs on sweet taste function, since early Poly detection was completely abolished when its activity at the SGLTs was blocked with PHL (*experiment 3*). Consistent with this, the sweet taste properties of Suc did not alone signal its immediate effects on intake, when its other preabsorptive and metabolic effects were impeded. Taken together, these results suggest that it is not just that intestinal sweet taste receptors have a higher affinity for sweet tastes in the form of carbohydrates over those of artificial sweeteners, but rather that the other stimulus effects of the carbohydrates, under normal conditions, gate sweet taste sensitivity in the intestine.

Insofar as sweet sensation from the tongue is elicited by most simple carbohydrates and some proteins (30, 31, 56), recasting these types of GI feedback signals as taste-like signals, in light of the newly discovered GI taste receptors, might seem relatively straightforward. However, the results from the present studies reveal that intestinal sweet taste sensitivity does not fit the gustatory template in a straightforward way. For one thing, naïve rats appeared to be relatively insensitive to a high concentration (20 mM) of Sucra alone at intestinal receptors, even after 10 pairings with LiCl in the present series, a finding that is consistent with previous work by Sclafani et al. (40), which showed that intragastric infusions of a high Sucra concentration failed to condition a flavor preference. By comparison, much lower concentrations of Sucra (~1.25–2.50 mM) at oral receptors are sufficient to guide intake behaviors (2, 26). Moreover, both in the present study (*experiment 1*) and Sclafani et al.'s study (40), a concentration of Suc (234 mM), selected to match 20 mM Sucra on the basis of oral preference thresholds (40), was successfully detected at postoral receptors to guide intake behavior. As discussed above, these differences in sensitivity to different ligands may reflect differences in the T1R dimerization on the tongue and in the stomach and intestines.

That said, it is clear from the posttraining generalization test data that postoral receptors are not altogether insensitive to 20 mM Sucra; responses to sweet taste alone were apparent after training with ID Suc or ID Sucra+ Poly in LiCl. Thus, alternatively, it may be the case that rather than directly transmitting sweet taste information about GI stimuli, postoral sweet taste receptors are modulated by other preabsorptive events. For example, the by-products of carbohydrate digestion (e.g., glucose) may engage apically positioned SGLTs and the downstream effects of these transceptors may feedback to activate GI T1Rs or otherwise alter the gain on GI T1R elicited

signals. In this sense, SGLTs might function much like sodium ion channels on the tongue, which have been shown to affect the sensitivity of the other receptors or signals generated by those other receptors within the same or nearby cell (49). Consistent with this putative mechanism, T1Rs are found interspersed among SGLT-positive cells in the epithelium of the GI tract (28). Furthermore, in this regard, it would be of interest to compare intestinal sweet taste detection following training with SGLT-dependent and -independent (e.g., fructose) stimuli. Continued work with the intestinal taste aversion paradigm with sweet taste alone (e.g., Sucralose) at various concentrations, with and without prior intestinal training with nutritive sweet tastants, will be useful in deciphering behaviorally relevant intestinal detection thresholds.

It is important to note that the carbohydrates used as training stimuli in *experiments 1* and *2* were additionally of higher osmolality than was the Sucra-alone training stimulus used in *experiment 1* (see Table 1). Thus we cannot altogether rule out the possibility that osmotic cues potentiated intestinal sweet taste detection under those more complex stimulus training conditions. However, acquisition appeared to be more rapid and the response more robust to the stimulus containing Poly compared with Suc, despite the fact that these solutions were of similar osmolality. Further suggesting that osmolality was not critical for ID sweet detection, in *experiment 3* those same osmotic cues failed to potentiate early responding to Suc and Poly, under conditions in which osmolality was relatively high, but normal carbohydrate digestion or transport was blocked. Whatever the case, it is clear that rats generalized from Suc, for example, to Sucra alone on the basis of intestinal sweet taste cues, not osmotic cues, at test (*experiments 1* and *2*).

Oral sweet taste receptors appear to be distinct from the putative intestinal receptors that respond to sweet insofar as oral T1Rs respond robustly to artificial sweet tastants on first exposure, and the oral tastes of nonnutritive stimuli are reinforcing in their own right (30, 40). However, there are some indications that oral sweet taste processing is also more complex and may include a synergistic mechanism, much like the one proposed here to underlie sweet taste detection in the GI tract. Adding glucose to an artificial sweet taste solution applied to the oral receptors results in a more robust signal than would be expected from a simple additive effect (16, 38, 44). This Sacc+glucose synergy is thought to happen as a result of the two ligands simultaneously binding at distinct loci on the oral T1R2+T1R3 complex, though this mechanism has been purely speculative to date (38). Recently, however, glucose transporters, including SGLT-1, have been identified in taste cells on the tongue, and, in some cases, are coexpressed with T1R3 (50, 55). Assuming these transporters and receptors interact functionally, they provide an alternative mechanism for these types of synergies. Moreover, repeated presentation of the compound sweet and carbohydrate stimulus paired with LiCl in the duodenum increased responding to an ID artificial sweet tastant alone, such that rats were responding to Sucra, in the absence of activated SGLT, for example, at test (*experiments 1* and *2*). It would be interesting to determine whether such lasting changes likewise occur following extensive experience of saccharin+ glucose mixtures at oral receptors.

At present, this hypothesis supposes that the potentiation of sweet taste is due to a specific relationship with the glucose transporters. As such, it would be expected that the combination of these particular stimuli would more successfully result in potentiation of the weak sweet taste in aversion learning, because of their coexpression and, speculatively, the positive interaction among their signals. Presumably though, any receptors that share these associations with sweet taste receptors ought to be equally successful. In particular, it would be of interest to determine whether this effect was due more to the sodium influx associated with glucose transport via SGLT in the intestine, or whether GLUTs work equally well. Of course, it is also necessary to preclude other strong stimuli, like bitters, or other caloric substances, like fats. The role of sweet taste receptors on the tongue is to identify and promote consumption of nutritive foods, as signified by their sweet taste properties. Even still, being expressly tuned to the presence of glucose within a sweet tasting substance may be metabolically meaningful. This specificity may even be more critical at the level of the intestine, where some nutrients (e.g., glucose) must be preferentially absorbed over others. Considering some of these putative similarities and differences in the molecular receptors and local signaling environments of oral and intestinal cells, future work ought to examine how experience with these sweet taste ligands at the intestinal receptors affects their later evaluation at oral receptors (51, 52).

Perspectives and Significance

To our knowledge, the present experiments are the first to demonstrate that sweet taste signals arising from the intestine are integrated for the behavioral control of ingestion. Previous attempts to link postoral sweet signals to higher order functions have failed to find evidence of such a connection. This is notably contrasted with the success of others in linking postoral sweet signals to physiological changes in the GI tract. In those physiological approaches, supplementation of an artificial sweet stimulus to a peri-threshold nutritive stimulus (e.g., glucose), low carbohydrate maintenance diet, or fed animal, increases transporter expression (27, 28, 46). SGLT-1 expression is blunted in α-gustducin- and T1R3 knockout mice (28). Although these effects have been taken to suggest GI sweet taste has a modulatory role in transporter regulation (21), the types of complex stimuli administered may enhance the sweet taste signal as well. Behaviorally speaking, presenting a robust stimulus (e.g., glucose) in compound with a weak stimulus (e.g., perhaps intestinal sweet taste) has been shown to increase responding to the weak component, beyond a simple additive effect (1). Indeed, the effects of postoral sweet taste on ingestive behavior in the present experiments were only evident following conditioning with a nutritive stimulus. Thus the apparent discrepancy between GI sweet taste's functional links to physiology versus behavior might be simply accounted for by the common use of more potent stimuli in the physiological work compared with that previously used in behavioral work.

Following the initial conditioning phase, simultaneous nutritive stimulation was not necessary for rats to suppress intake in response to the sweet cue arriving in the intestine. Such experience-induced changes in sensitivity have been demonstrated in the both oral taste system and other GI signaling systems (6, 35). For example, chronic exposure to a high-fat diet decreased sensitivity to the gut satiety hormone CCK and led to overconsumption (35). Future work will need to explore the conditions necessary to produce these types of interactions,

with respect to implications for both local GI signals and centrally mediated signals.

Together, the emerging evidence on the role of postoral sweet taste signaling in glucose absorption and incretin release has been noted for its implications in developing new treatment strategies for diabetes (e.g., 54). The behavioral data from this present series highlight two additional points for consideration. First, sweet taste may only be sufficiently strong to elicit these changes under conditions in which glucose is also present in the GI tract. Second, in the event that GI sweet taste stimulation is sufficient to affect GI transporter expression or incretin release, the signal may still be too weak to be integrated into satiety and other centrally mediated effects related to glucose regulation and homeostasis, without glucose present or without specific extensive exposure (and reexposure) with glucose (and related compounds).

The circumscribed response patterns in the intestinal taste aversion paradigm will greatly benefit future work aimed at deciphering the contributions of specific taste receptors (e.g., with taste receptor knockout mice) and afferent pathways (e.g., with vagal deafferentations) in the GI tract. Moreover, given that the primary signaling molecules of the intestine are hormones, which can act both as rapid paracrine factors on local afferent terminals and as longer term endocrine factors at various peripheral and central targets, paradigms or testing conditions that, for example, pretreat the animal with a specific hormone receptor antagonist (e.g., GLP-1R) and measure cumulative intake over a period of 30 min or more are rather limited with respect to deciphering which pathway the signal employs to affect behavior. In fact, these distinctions (paracrine, endocrine) may be particularly important in developing more effective pharmacological or other interventions in the control of food intake.

ACKNOWLEDGMENTS

The authors thank Drs. Edward Fox and Kimberly Kinzig for helpful comments on these experiments and Tom Karam and Adella Birge for assistance with animal care. This report contains part of a PhD thesis by L. A. Schier. A portion of this report appeared in abstract form at the 19th Annual Meeting of the Society for the Study of Ingestive Behavior, 2011.

GRANTS

This work was supported by National Institutes of Health Grants R01 DK-027627 and P01-HD-05211.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: L.A.S. conception and design of research; L.A.S. performed experiments; L.A.S. analyzed data; L.A.S., T.L.D., and T.L.P. interpreted results of experiments; L.A.S. prepared figures; L.A.S. drafted manuscript; L.A.S., T.L.D., and T.L.P. edited and revised manuscript; L.A.S., T.L.D., and T.L.P. approved final version of manuscript.

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