

Consumption of SC45647 and Sucralose by Rats Selectively Bred for High and Low Saccharin Intake

Nancy K. Dess, Clinton D. Chapman and Derek Monroe

Department of Psychology, Occidental College, 1600 Campus Road, Los Angeles, CA 90041, USA

Correspondence to be sent to: Nancy K. Dess, Department of Psychology, Occidental College, 1600 Campus Road, Los Angeles, CA 90041, USA. e-mail: dessnk@oxy.edu

Abstract

Mammals' affinity for sweet tastes exists alongside dramatic variation among species and individuals in responses to sweeteners. The present paper focused on consumption by Occidental High- (HiS) and Low-Saccharin (LoS)-consuming rats in 23-h 2-bottle tests of 2 sweeteners for which few data from rats are available: SC45647 and sucralose. Every HiS and LoS rat preferred SC45647 to water at every concentration, with HiS rats consuming it more avidly. Most HiS rats preferred sucralose to water at one or more concentrations; some HiS rats and most LoS rats avoided sucralose at every concentration. However, both HiS and LoS rats preferred a sucralose–maltodextrin mixture (Splenda) to water; thus, Splenda's "bulking" ingredient maltodextrin transforms highly variable responses to sucralose into a relatively homogeneous preference for the product. Implications for the study of variation in sweet taste are discussed.

Key words: maltodextrin, rats, SC45647, selective breeding, Splenda, sucralose

Introduction

The pervasive behavioral affinity among mammals for sweet tastes makes sense in terms of the adaptive advantage of detecting and preferring carbohydrates. Yet, the sweet taste world is remarkably diverse, with preference for many substances perceived as sweet by humans varying dramatically across and within species (see reviews by Hellekant and Danilova 1996; Mennella et al. 2005; Hayes 2008). For instance, hamsters, mice, rats, lemurs, rhesus monkeys, and chimpanzees all prefer sugars and saccharin to water; on the other hand, hamsters, mice, rats, and lemurs show little to no preference for aspartame over water, whereas rhesus monkeys and apes prefer it (Sclafani and Abrams 1986; Glaser et al. 1995; Danilova et al. 1998; Bachmanov et al. 2001; Schilling et al. 2004). These findings tempt a simple phylogenetic story of sweet taste. In this tale, a common mammalian ancestor equipped to prefer sugars and saccharin (but not aspartame) gave rise to similarly endowed rodents and New World primates from whom aspartame-preferring Old World primates parted gustatory ways.

The simplest story is, of course, incomplete (Glaser 2002; Hayes 2008). The common marmoset, a New World monkey, prefers neither aspartame nor saccharin to water (Danilova and Hellekant 2004), whereas fruit flies—like Old World monkeys and apes—prefer both to water (Gordesky-Gold

et al. 2008). Moreover, phenotypic similarity between related species does not always derive from shared mechanisms. For example, *Tas1r3* polymorphisms linked to variation across mouse strains in saccharin preference (Reed et al. 2004) do not account for saccharin phenotype variation among rats (Lu et al. 2005), the expression of which increases between weaning and adulthood (Carroll et al. 2008). Clearly, multiple evolutionary and epigenetic processes shape sweet taste repertoires, and much remains to be learned about sources of phenotypic variation in sweet taste.

Rats remain integral to this endeavor, but little is known about their response to the noncarbohydrate sweeteners SC45647 and sucralose. These sweeteners have been studied more extensively in mice and nonhuman primates (e.g., Bachmanov et al. 2001; Danilova and Hellekant 2004; Schilling et al. 2004; Inoue et al. 2007), and enhancing the database for rats will help establish whether the findings hold generality or are specific to species or strains. A literature search reveals 3 studies bearing on rats' response to SC45647. In 2 of them (Heyer et al. 2003, 2004), conditioning procedures indicated that SC45647 had a sweet taste quality, but 2-bottle preference tests were not conducted. Only one study included data on voluntary consumption. Gosnell et al. (1998) measured intake of SC45647 (17 mg/L) during

23-h 2-bottle tests in 33 male Harlan Sprague Dawley rats. SC45647 intake exceeded water intake and was positively correlated with saccharin intake. SC45647 is preferred to water by mice and hamsters (Bachmanov et al. 2001; Schilling et al. 2004), but behavior toward sweet taste and its mechanisms can differ even between closely related species (Danilova and Hellekant 2004; Lu et al. 2005). Therefore, replication of measurement of Gosnell et al. (1998) at several concentrations, with both males and females, would be useful.

Little research on sucralose has been done with rats. Sclafani and Clare (2004) reported overall indifference to sucralose (0.25–4.0 g/L) in female Charles River rats. Responses were highly variable, with about half of the rats preferring sucralose at 0.5 g/L and most other concentrations and the other half avoiding it at all concentrations. Sucralose preference was rarer among males, with only 7 of 42 male rats preferring it (reported only for 0.5 g/L; p. 527). The authors concluded that sucralose has an aversive side taste that varies with concentration and among individuals. Bello and Hajnal (2005) similarly reported that only 3 of 13 male Charles River rats preferred 0.5 g/L sucralose to water. In those studies, sucralose preference appears to be stronger among female than male rats. However, the difference may have been due to the small samples or the particular strain used in both studies.

The available evidence suggests that rats' perception of sucralose is qualitatively similar to their perception of saccharin (Dess 1993). Responses to both tastants range from preference to aversion and are relatively stable within individuals, with preference decreasing and/or aversion increasing at higher concentrations. These findings suggest that, like saccharin, sucralose has hedonically mixed taste qualities, responses to which distinguish subpopulations of rats; more rats seem to be truly averse to sucralose than to saccharin. Whether bitterness is the aversive side taste of sucralose for rats is not clear; however, sucralose is a chlorinated sugar (trichlorogalactosucrose), for which bitterness is a common taste quality (Shamil et al. 1987; Mathlouthi and Hutteau 1999).

In one sense, the notion that sucralose is fundamentally bitter–sweet is unsurprising: many noncarbohydrate sweeteners have a bitter or other aversive side taste—long the bane of entrepreneurs seeking a nonnutritive sweetener that tastes as good as sugar. In another sense, sucralose's hedonically mixed taste makes the success of the sucralose-containing product Splenda seem paradoxical. Sucralose's taste—touted as “all sweetness and light” (Binns 2003)—seems an obvious reason for Splenda having captured two-thirds of the \$1.5 billion nonnutritive sweetener market in the United States (Browning 2007). Are people insensitive to an aversive side taste that rats detect? That does not appear to be so. Like saccharin and unlike sugars, humans' sucralose bitterness ratings increase with concentration; its bitterness exceeds that of sucrose, glucose, and fructose at higher concentrations for which sweetness is comparable (Schiffman et al.

1995). Thus, although sucralose may be more palatable to humans than to rats, humans do report a bitter side taste, albeit one that is less intense than saccharin's and is minimal at low concentrations (Wiet and Beyts 1992). Moreover, data in those studies were aggregated, and the variability around mean bitterness ratings may in part reflect variation among individuals in sucralose's bitterness (Kamerud and Delwiche 2007). The present study bears on the possibility that a discrepancy between the taste of sucralose and Splenda could contribute to the latter's broad appeal.

According to the Splenda Consumer Relationship Center, granular Splenda is 93% maltodextrin by weight. The term “maltodextrin” refers to a diverse group of glucose polymer mixtures produced by hydrolyzing starch. Maltodextrins contain different mixtures of longer and shorter chain-length glucose polymers and sugars (glucose and disaccharides) depending on the starch source and manufacturing process (Roller 1996). The maltodextrin Polycose, for example, consists of polymers with chain lengths mostly in the 3–8 range, along with approximately 10% sugars (glucose and maltose) and 30% starch (Abbott Nutrition, Ross Products Division, personal communication; Kennedy et al. 1985; Quezada-Calvillo et al. 2007). Rats prefer maltodextrin to water (Sclafani and Nissenbaum 1987; Sclafani et al. 1998; Davis and Breslin 2000). Maltodextrin's palatability does not derive entirely from starch—which rats do distinguish from sugar and Polycose and prefer to water (Nissenbaum and Sclafani 1987; Ramirez 1991)—because maltodextrin's palatability is greater when shorter, not longer, chain-length polymers predominate (Sclafani, Hertwig, et al. 1987; Ramirez 1994). Maltodextrin might enhance Splenda's palatability by masking sucralose's aversive side taste, additively increasing the palatability of the mixture or interacting with its taste in some way.

Whether the same is as likely for humans is less clear because limited and mixed human psychophysical results are available on maltodextrin's taste. Hettinger et al. (1996) found that 5 of 10 people reported a sweet quality for Polycose solution at 3% weight/volume; olfaction mediated that quality, as nobody reported it during nose-closed sampling. At 10% Polycose, the number reporting a sweet quality rose to 8 with or without noses closed and few reported any of 5 aversive taste qualities. In contrast, Feigin et al. (1987) reported that Polycose was not generally described as sweet or pleasant at 3–10% and was described as sweet but unpleasant at higher concentrations. However, pleasantness varied considerably among participants, and sucrose solution also was not described as pleasant. In fact, in 1 of 2 experiments, the pleasantness of Polycose and sucrose did not differ significantly. As the authors note, these findings illustrate the context specificity of tastants' palatability: a tastant capable of enhancing the palatability of complex solutions or substances does not necessarily make a tasty aqueous solution by itself. Thus, even if maltodextrin is not unequivocally sweet or palatable, it could enhance the palatability of

Splenda and beverages or foods to which it is added, perhaps more for some people than for others.

To date, no research has compared responses to sucralose with responses to Splenda in rats or people. The present study allowed comparison of rats' consumption of sucralose versus water in 2-bottle tests to consumption of Splenda versus water in 2-bottle tests. The only ingredient other than sucralose in granular Splenda is maltodextrin (McNeil Nutritionals LLC), so any difference in results for sucralose and Splenda must be due to maltodextrin. Sucralose's aversive taste and maltodextrin's palatability may be more pronounced in the average rat than in the average person, but clear evidence that sucralose tastes different than Splenda to rats would provide a preliminary basis for speculating that maltodextrin could influence Splenda's palatability in people.

The present study adds to the research on how rats respond to SC45647 and sucralose by examining voluntary consumption in rats selectively bred on a taste phenotype. The Occidental High-Saccharin (HiS) and Low-Saccharin (LoS)-consuming lines have been selectively outbred on the basis of extreme scores on amount of sodium saccharin solution (1.0 g/L) consumed in a 23-h 2-bottle test (see details in Carroll et al. 2008). The saccharin intake selection phenotypes have been stable for 20 generations. In addition to saccharin, HiS rats consume dilute sucrose, glucose, maltose, fructose, Polycose, and sodium chloride more avidly than do LoS rats (Dess 2000). Most LoS rats drink more saccharin solution than water at lower concentrations (0.5–1.0 g/L), but they consume it less avidly and have a lower aversion threshold than HiS rats (Dess and Minor 1996). Overall, both lines slightly (albeit significantly) prefer aspartame to water, but neither preference nor amount consumed differs between lines (De Francisco and Dess 1998).

HiS and LoS rats' differential intake of palatable tastants is not mirrored in aversion to purely aversive tastants (Dess 2000). The lines do not differ in aversion to bitter quinine or sucrose octaacetate solutions or to sour citric acid solution. Nor do the lines differ in sensitivity to adulteration of sucrose solution with citric acid. However, LoS rats reject sucrose solution adulterated with quinine at a lower quinine concentration. Their greater responsiveness to the bitter component of a bitter-sweet taste probably contributes to their relatively low intake of ethanol and saccharin (Dess et al. 1998).

Work continues on determining whether these functional line differences reflect variation at the taste receptor, brain stem, and/or forebrain level. HiS and LoS rats are not distinguished by protein-coding regions of *Tas1r3* that distinguish mouse strains that consume different amounts of saccharin (Lu et al. 2005). Although genes other than *Tas1r3* or taste receptors other than T1R3 could explain the line differences, the likelihood of finding an explanation at the taste receptor level is further reduced by the fact that the lines do not differ in taste reactivity to saccharin in an initial 5-min exposure; LoS rats display greater aversive taste reactivity to saccharin only upon reexposure (Badia Elder et al. 1995;

Thiele et al. 1997). The role of taste experience and post-weaning development (Carroll et al. 2008) in expression of the saccharin phenotype implicates higher order influences on taste in the line difference, including mechanisms related to learning, reward, and risk reactivity (Dess and Minor 1996; Dess et al. 2008). The implication of such mechanisms suggests that a "bottom-up" explanation starting at the tongue is unlikely to suffice. Testing HiS and LoS rats with a broader range of chemically different sweeteners for which data are available in other strains and species will help in the development of a more comprehensive explanation of individual differences in sweet taste.

In the present study, HiS and LoS rats were given SC45647 (Experiment 1), sucralose in 2 concentration ranges (Experiments 2 and 3), and Splenda (Experiment 4) in 23-h 2-bottle tests. Tastant concentrations were chosen with the goals of 1) avoiding subthreshold or saturation concentrations and 2) overlapping with concentrations used in other studies. The methods did not allow identification of detection or preference/aversion thresholds for all groups. However, they did allow us to compare HiS and LoS rats, to compare females and males, and to compare these results to those from other laboratories.

Materials and methods

Animals

Experimentally naive female and male HiS and LoS rats (average 88 days of age, range 62–145 days of age) representing 4–8 litters in each line from generations 23 to 34 were used in each experiment. Rats were housed individually with continuous access to Purina 5001 rodent chow and tap water on a 12:12 light:dark cycle (light onset at 0700). Care and use of the rats complied with the institution's Public Health Service Assurance.

Tastant solutions

Solutions were made daily with tap water. SC45647 (courtesy of G. Hellekant) solutions were 4, 8, and 16 mg/L (Experiment 1). Sucralose (courtesy of Tate & Lyle Inc., Decatur, IL, and A. Sclafani) was tested in 2 concentration ranges. The concentrations in Experiment 2 (0.25, 0.5, 0.75, and 1.0 g/L) overlapped with concentrations used by Sclafani and Clare (2004). Lower concentrations (0.01, 0.02, 0.05, and 0.1 g/L) were used in Experiment 3 for 2 reasons. First, the aversion among LoS rats at all concentrations in Experiment 2 left open the possibility that they would prefer sucralose at lower concentrations. Second, the lower concentrations corresponded roughly to the concentrations of sucralose in the Splenda solutions used in Experiment 4. Those Splenda concentrations were 0.12, 0.24, 0.5, 0.6, 1.0, and 2.0 g/L, which, according to manufacturer information on sweetness equivalence for humans, correspond to about 1–20 g/L sucrose solution. Boxed granular Splenda was purchased

from the manufacturer (McNeil Nutritionals LLC, Fort Washington, PA).

Procedure

Body weight and water intake were measured during a 2-day baseline period. Rats then received a series of 23-h 2-bottle tests with a tastant solution and tap water. Tastant solutions were presented in a 250- or 500-mL glass bottle, and water was presented in a 50-mL polypropylene tube or 125-mL glass bottle. HiS and LoS rats prefer water in glass and in polypropylene equally (Dess and Minor 1996). Bottles and tubes had rubber stoppers with stainless steel spouts. Left–right positions of tastant solution and water were balanced across rats and alternated across test days.

In Experiments 1–3, tastant concentrations were presented once each, in ascending order on consecutive days. In Experiment 4, rats first received Splenda at 0.6 g/L for 1 day. Due to an unexpectedly high preference at this concentration this test was followed by a 23-h test at 2.0 g/L, comprising a pre-exposure phase; then, after 1 day of water only, the rats were tested with 0.12–2.0 g/L Splenda in ascending order on consecutive days.

Data analysis

Studies to date have revealed no consistent line differences in preexperimental body weight or water intake. Occasionally, high baseline water intake is observed. Because such animals may differ from others in their ability to regulate hydration, males whose average daily water intake exceeded 20% of their body weight and females whose average daily water intake exceeded 30% of their body weight were eliminated from this study. Applying this criterion resulted in elimination of 2 HiS females in Experiment 1, 2 HiS males and 1 HiS female in Experiment 2, 1 LoS male and 1 HiS female in Experiment 3, and no rats in Experiment 4.

Each baseline measure was subjected to a line \times sex analysis of variance (ANOVA). Fluid intake in each experiment was subjected to an ANOVA with line, sex, solution (tastant vs. water), and concentration as variables; Greenhouse-Geisser-corrected P values were used to evaluate concentration effects. The highest order interactions involving any variable were interpreted with pairwise contrasts, using Bonferroni adjustment to control Type I error rate.

Supplemental analyses were performed to permit direct comparison of these results to other studies. In Experiment 1, data from HiS and LoS rats at the highest SC45647 concentration (16 mg/L) were transformed for comparison to results of Gosnell et al. (1998) for males tested at 17 mg/L SC45647. Transformations included preference scores (grams of SC45647 per total fluid grams; mean and standard error mean [SEM] were reported) and avidity, a measure of volume consumed relative to water baseline (SC45647 solution grams divided by water baseline grams; range was reported). Each measure was subjected to a line \times sex ANOVA.

In Experiment 2, sucralose preferrers and nonpreferrers were identified by applying definitions used by Sclafani and Clare (2004). Preference scores were calculated at each concentration for each rat. Sucralose “preferrers” were rats with a preference score above 0.50 at any concentration and “nonpreferrers” were all other rats. Whether the proportion of preferrers to nonpreferrers differed between lines and/or sexes was evaluated with chi-square tests. These tests were repeated on data from only 0.5 g/L sucralose to allow direct comparison to results at that concentration reported by Sclafani and Clare (2004) and Bello and Hajnal (2005). Finally, a frequency distribution was constructed from preference scores averaged across all concentrations for each rat so that its shape could be examined for bimodality.

Significant test statistics with $P < 0.05$, 0.01, or 0.001 are reported in the text. For other results, $P > 0.05$.

Results

Baseline body weight and water consumption

Average body weight and water consumption for rats meeting the inclusion criterion are shown in Table 1. In each experiment, female rats weighed less ($F_s > 100$) and drank less water ($F_s > 6$) than males, and neither body weight nor water intake differed between lines overall. In Experiment 3, a line \times sex interaction was observed for body weight [$F(1,38) = 19.69$, $P < 0.001$]: female LoS rats were heavier than female HiS rats, and male LoS rats were lighter than male HiS rats. This difference was due to the female LoS rats being older than the HiS female rats (118 vs. 90 days of age) and the male LoS rats being slightly (not significantly) younger than the male HiS rats (75 vs. 82 days of age) [line \times sex interaction, $F(1,38) = 10.68$, $P < 0.01$]. Age differences were not significant in the other experiments.

Experiment 1: SC45647

Intake of SC45647 solution and water is shown in Figure 1. Average baseline water intake is shown for comparison. All groups drank more SC45647 solution than water. In fact, every rat drank more SC45647 solution than water at every concentration. SC45647 intake increased with concentration, more so among HiS rats. The ANOVA yielded main effects of solution [$F(1,40) = 867.03$, $P < 0.001$], concentration [$F(3,80) = 49.88$, $P < 0.001$], and line [$F(1,40) = 68.15$, $P < 0.001$]; a solution \times concentration interaction [$F(2,80) = 49.28$, $P < 0.001$]; and 3 interactions involving line: line \times solution [$F(1,40) = 78.47$, $P < 0.001$], line \times concentration [$F(2,80) = 17.07$, $P < 0.001$], line \times solution \times concentration [$F(2,80) = 14.78$, $P < 0.001$]. Bonferroni-adjusted contrasts showed that HiS rats drank more SC45647 than LoS rats at each concentration, and the lines did not differ on water intake at any concentration. No effects involving sex were significant, reflecting females' general tendency to consume

Table 1 Initial body weight and 24-h water consumption, both expressed as grams (mean \pm SEM)

	Body weight	Water intake (g)
Experiment 1		
HiS female ($n = 9$)	259 \pm 2 ^b	50 \pm 5 ^b
HiS male ($n = 12$)	457 \pm 12 ^a	55 \pm 3 ^a
LoS female ($n = 12$)	272 \pm 10 ^b	45 \pm 2 ^b
LoS male ($n = 11$)	434 \pm 15 ^a	56 \pm 3 ^a
Experiment 2		
HiS female ($n = 11$)	249 \pm 11 ^b	45 \pm 3 ^b
HiS male ($n = 10$)	377 \pm 15 ^a	59 \pm 4 ^a
LoS female ($n = 12$)	260 \pm 9 ^b	43 \pm 2 ^b
LoS male ($n = 12$)	390 \pm 14 ^a	53 \pm 2 ^a
Experiment 3		
HiS female ($n = 10$)	264 \pm 8 ^c	44 \pm 2 ^b
HiS male ($n = 10$)	424 \pm 14 ^a	54 \pm 3 ^a
LoS female ($n = 12$)	329 \pm 7 ^b	44 \pm 2 ^b
LoS male ($n = 10$)	364 \pm 23 ^b	53 \pm 2 ^a
Experiment 4		
HiS female ($n = 14$)	268 \pm 8 ^b	41 \pm 2 ^b
HiS male ($n = 17$)	482 \pm 12 ^a	57 \pm 3 ^a
LoS female ($n = 16$)	300 \pm 10 ^b	45 \pm 2 ^b
LoS male ($n = 14$)	470 \pm 14 ^a	58 \pm 2 ^a

Groups with different superscript letters differed significantly ($P < 0.05$).

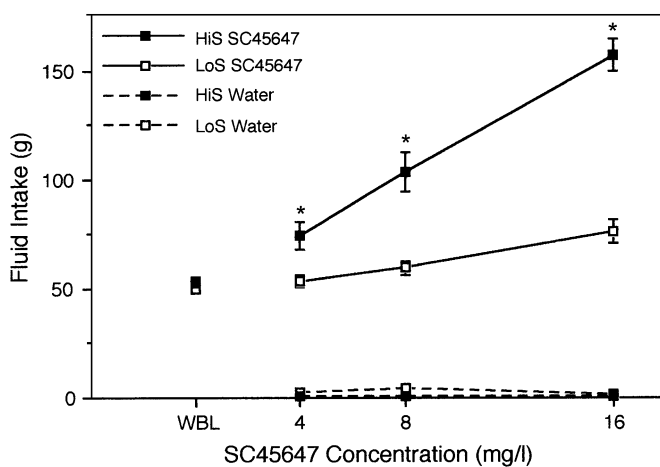


Figure 1 Experiment 1: Intake (grams) of SC45647 solution and water (mean \pm SEM) for HiS and LoS rats in 23-h 2-bottle tests. Means are collapsed across sexes. Average daily baseline water intake (WBL) is shown for comparison. Asterisks indicate that the line difference for SC45647 was significant at each concentration (HiS > LoS).

more fluid relative to their body weight than age-matched males do (e.g., Dess 2000).

The preference score at 16 mg/L averaged across all rats was 0.98 ± 0.01 (mean \pm SEM), compared with 0.91 ± 0.03 at 17 mg/L reported by Gosnell et al. (1998). Avidity scores ranged from 0.66 to 5.15, compared with 0.63–2.40 reported by Gosnell et al. The line \times sex ANOVA on preference scores yielded no significant effects. The line \times sex ANOVA on avidity scores yielded main effects of line [HiS, 3.14 ± 0.23 vs. LoS, 1.55 ± 0.12 ; $F(1,40) = 49.04$, $P < 0.001$] and sex [females, 2.56 ± 0.29 vs. males, 2.07 ± 0.20 ; $F(1,40) = 7.62$, $P < 0.01$].

In sum, HiS and LoS rats strongly preferred SC45647 to water, and amount of SC45647 consumed was predicted by saccharin phenotype (HiS > LoS). These results conform well with the prior data from only males (Gosnell et al. 1998), though the highest avidity scores among HiS and female rats were much higher than observed in the prior study.

Experiment 2: sucralose (0.25–1.0 g/L)

Sucralose solution and water intake are shown in Figure 2. LoS rats avoided sucralose, and HiS rats drank more sucralose than water. The ANOVA yielded a main effect of line [HiS > LoS, $F(1,41) = 23.01$, $P < 0.001$]. Two interactions were interpreted with contrasts. The solution \times concentration interaction was significant [$F(3,123) = 3.71$, $P < 0.05$]; sucralose solution intake exceeded water intake reliably only at the second lowest concentration. Solution also interacted with line [$F(1,41) = 39.36$, $P < 0.001$]; HiS rats drank more sucralose solution than LoS rats did, whereas LoS rats drank more water than HiS rats did. No effects involving sex were significant.

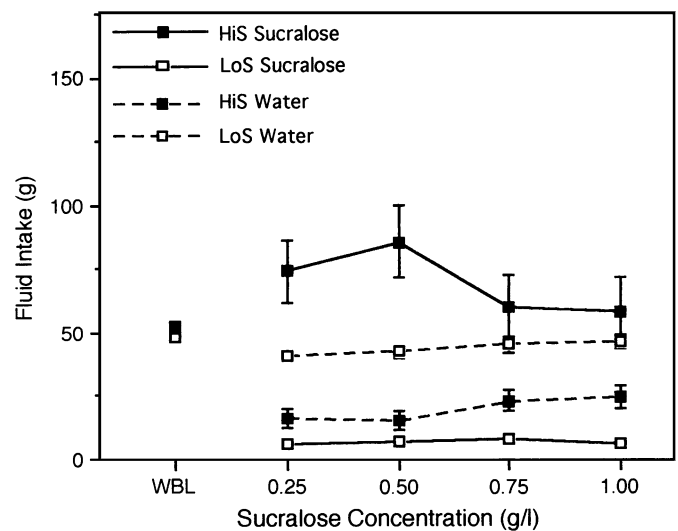


Figure 2 Experiment 2: Intake (grams) of sucralose solution and water (mean \pm SEM) for HiS and LoS rats in 23-h 2-bottle tests. Means are collapsed across sexes. Average daily baseline water intake (Water Baseline, WBL) is shown for comparison. The overall line differences in sucralose (HiS > LoS) and water (LoS > HiS) intake were significant and did not vary with concentration.

To compare our results directly to the findings of Sclafani and Clare (2004) with females, preferer/nonpreferer status was tallied by line for each sex. Overall, about half of the females were preferers (12 of 23). Most LoS females (9 of 12) were nonpreferers, whereas most HiS females (9 of 11) were preferers [$\chi^2(1) = 7.42, P < 0.01$].

The results were similar for males. Nine of the 22 males were preferers. Most LoS males (11 of 12) were nonpreferers, whereas most HiS males (8 of 10) were preferers [$\chi^2(1) = 11.59, P = 0.001$]. A separate preferer status \times sex chi-square test showed no difference in the proportions of preferers and nonpreferers for females and males.

Preferer/nonpreferer analyses on data for 0.5 g/L sucralose yielded identical results for males (8 of 10 HiS rats preferers and 11 of 12 LoS rats nonpreferers). Among females, somewhat fewer were preferers than when all concentrations were considered (7 of 23). All 7 were HiS rats, whereas all 12 LoS rats were nonpreferers [$\chi^2(1) = 10.98, P < 0.001$].

Because preferer/nonpreferer dichotomy of Sclafani and Clare (2004) splits the rats into 2 roughly equal groups, they referred to the sucralose preference as bimodal. However, their analysis did not speak directly to whether the distribution was bimodal. This question was addressed for LoS and HiS rats by constructing a frequency distribution of preference scores. Females and males, which did not differ, were pooled. The result (Figure 3) clearly indicates a bimodal

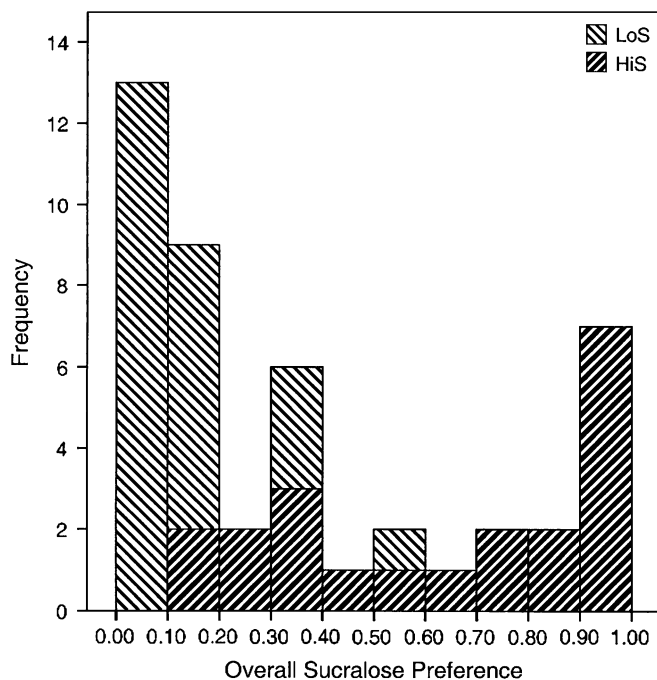


Figure 3 Frequency distribution for sucralose preference (0.50 = indifference), averaged across concentrations for each rat. Males and females did not differ and are not distinguished here. Counts for LoS (light right hashmarks) and HiS (heavy left hashmarks) rats are stacked so that the shape of the overall distribution can be discerned.

distribution, with most LoS rats averse to sucralose and most HiS rats preferring it.

Thus, as reported by others, rats tended to either prefer or avoid sucralose. The overall proportion of preferers to nonpreferers was comparable in females and males, and the proportion of preferers at 0.5 g/L was somewhat smaller for females than for males. The relatively smaller percentage of male preferers described by Bello and Hajnal (2005) and Sclafani and Clare (2004) likely reflected either unrepresentative samples or a strain difference.

Experiment 3: sucralose (0.01–0.1 g/L)

In Experiment 2, LoS rats drank little sucralose at all concentrations, leaving open the possibility that they would prefer sucralose at lower concentrations. Results for the lower concentrations used in Experiment 3 are depicted in Figure 4. At the lowest concentration, no line difference is apparent. As concentration increased, HiS rats consumed increasingly more sucralose solution and LoS rats consumed increasingly more water. The ANOVA yielded main effects of line [HiS > LoS, $F(1,38) = 7.88, P < 0.01$] and solution [sucralose > water, $F(1,38) = 14.06, P = 0.001$]. Three interactions involving line were significant: line \times solution [$F(1,38) = 55.09, P < 0.001$], line \times concentration [$F(3,114) = 3.50, P < 0.05$], and line \times solution \times concentration [$F(3,114) = 25.68, P < 0.001$]. Contrasts showed that neither sucralose nor water intake differed between lines at the lowest concentration; at each higher concentration, HiS rats drank more sucralose solution than LoS rats and LoS rats drank more water than HiS rats. This pattern indicates comparable preference and aversion thresholds in, respectively, HiS and LoS rats.

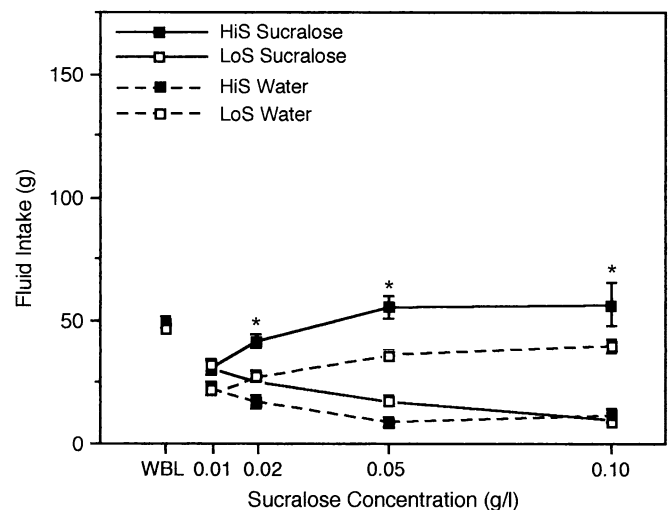


Figure 4 Experiment 3: Intake (grams) of sucralose solution and water (mean \pm SEM) for HiS and LoS rats in 23-h 2-bottle tests. Means are collapsed across sexes. Average daily baseline water intake (WBL) is shown for comparison. Asterisks indicate the concentrations at which the line differences in sucralose (HiS > LoS) and water (LoS > HiS) intake were significant.

Neither the main effect of sex nor interactions involving sex were significant. Thus, the observed line differences in fluid intake cannot be explained in terms of body weight or age, which differed significantly only between LoS and HiS females.

Experiment 4: Splenda

In the initial tests of Splenda at 0.6 and 2 g/L (left panel, Figure 5), rats drank more Splenda solution than water. This difference increased with concentration, more so for HiS rats. Male rats drank more than females, with a bigger difference for Splenda than for water. The ANOVA yielded main effects of solution [$F(1,57) = 635.85, P < 0.001$], concentration [$F(1,57) = 37.61, P < 0.001$], and sex [male > female, $F(1,57) = 5.14, P = 0.03$]. The solution \times sex interaction was significant [$F(1,57) = 7.17, P = 0.01$]; males drank more Splenda solution than females, and the sexes did not differ in water intake. Other interactions were solution \times line [$F(1,57) = 6.87, P = 0.01$], solution \times concentration [$F(1,57) = 51.67, P < 0.001$], and solution \times concentration \times line [$F(1,57) = 8.62, P = 0.005$]. Contrasts showed that HiS rats drank more Splenda solution than LoS rats at the higher concentration; the lines did not differ for Splenda at the lower concentration or for water at either Splenda concentration.

Results for the subsequent tests (right panel, Figure 5) were essentially the same. The ANOVA yielded main effects of solution [$F(1,57) = 582.97, P < 0.001$], concentration [$F(4,228) = 39.30, P < 0.001$], and sex [male > female, $F(1,57) = 15.23, P < 0.001$]. Significant interactions were solution \times sex [$F(1,57) = 13.15, P = 0.001$], solution \times concentration [$F(4,228) = 46.80, P < 0.001$], and solution \times concentration \times line [$F(4,228) = 5.59, P = 0.001$]. HiS rats drank significantly more Splenda solution than LoS rats only at the

highest concentration; the lines did not differ in water intake on any test.

Discussion

The present study extends previous behavioral studies of sweet taste in a number of ways. The preference for SC45647 and correlation with saccharin intake reported by Gosnell et al. (1998) for male rats were replicated and extended to lower concentrations and to females. The uniformity and strength of the LoS rats' preference for SC45647 is notable in light of their enhanced sensitivity to bitter components in complex tastes (Dess 2000). Saccharin and many other nonnutritive sweeteners have a bitter taste for at least some individuals, and the bitterness tends to increase with concentration (Dess 1993; Schiffman et al. 1995). On these bases, we might have expected at least some LoS rats to be averse to SC45647. The uniform preference for SC45647 suggests that bitter or other side tastes of SC45647, if any, are weaker than for saccharin.

Sucralose provides a striking contrast with SC45647 and saccharin. Intake of the most preferred sucralose concentrations (0.25–0.50 g/L) was comparable to intake of the least preferred SC45647 concentration (4 mg/L). At the most preferred concentrations of the 2 compounds, a typical HiS rat consumed twice as much SC45647 as sucralose; intake of 16 mg/L SC45647 was comparable to what is typically observed for the 1-g/L saccharin solution used to assess the selection phenotype (Dess and Minor 1996; Carroll et al. 2008). Also, whereas nearly all HiS and LoS rats prefer SC45647 and saccharin to water at most concentrations, sucralose was avoided altogether by many rats in both lines. Most sucralose nonpreferrers were LoS rats, and most preferrers were HiS rats. These results suggest that responses to sucralose and the saccharin intake phenotype share a common source of variation. In the study of Sclafani and Clare (2004), trends were in the direction of less saccharin intake among sucralose nonpreferrers. Those trends were not statistically significant, but the preferer and nonpreferer subgroups included only 6 rats each, limiting statistical power. Whether small sample size or something else accounts for relationship between sucralose and saccharin intake being weaker in their study than in the present one or Gosnell et al. (1998) remains to be determined.

Sucralose's aversive taste component might prevent HiS rats from consuming it more avidly than they do, but the degree to which most LoS rats reject sucralose is remarkable. The proportion of LoS rats frankly averse to sucralose greatly exceeds what we observe for saccharin at low to moderate concentrations (about 1 in 10 rats) and contrasts sharply with HiS rats' general preference for sucralose. These results reinforce the notion that sucralose's taste is hedonically complex and highly variable among rats (Sclafani and Clare 2004). A bitter side taste seems likely, as HiS and LoS rats differ in aversion to sweet solution adulterated with

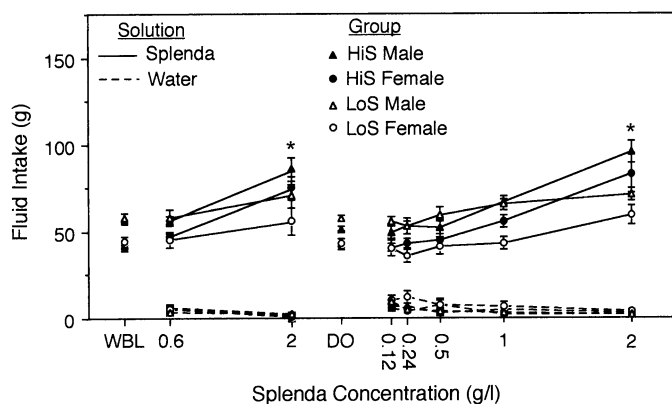


Figure 5 Experiment 4: Intake (grams) of Splenda solution and water (mean \pm SEM) for male and female HiS and LoS rats in 23-h 2-bottle tests. Average daily water intake (WBL) and average water intake on the "day off" between test series (DO) are shown for comparison. Asterisks indicate that the line difference in Splenda intake (HiS > LoS) was significant at the highest concentration.

a bitter but not a sour tastant (Dess 2000). Confirmation that the aversive side taste is bitterness requires study with other techniques, such as conditioned taste aversion generalization or operant discrimination tasks. Ironically, LoS rats' robust aversion to sucralose implies that it activates the sweet taste system at some level because LoS rats do not differ from HiS rats in response to purely aversive tastants such as quinine, sucrose octaacetate, or citric acid (Dess 2000). Nonetheless, such activation is insufficient to produce among LoS rats the inverted-U preference/aversion curve observed for saccharin (Dess and Minor 1996).

Whatever sucralose's aversive side taste is for rats, mice might not detect it. Bachmanov et al. (2001) measured amount consumed and preference and aversion thresholds for 129P3/J (129) and C57BL/6ByJ (B6) mouse strains with a wide variety of sweeteners, including sucralose, SC45647, and saccharin. Results for SC45647 and saccharin were consistent with the present findings and other research: both strains preferred SC45647 to water up to the highest concentration tested (300 mg/L), and both strains preferred saccharin at lower concentrations and were averse to it at high concentrations. The 129 mice consumed SC45647 and saccharin less avidly than B6 mice and had a lower aversion threshold for saccharin; in these respects, 129 mice resemble LoS rats and B6 mice resemble HiS rats. However, both mouse strains preferred sucralose to water at up to the highest concentration tested (10 g/L). Moreover, at and above the highest sucralose concentration used in the present study, every mouse in both strains preferred sucralose to water (Bachmanov, AA personal communication). The present and previous findings of aversion to sucralose in a substantial subpopulation of rats from different strains support the inference that, as Sclafani and Clare (2004) speculated, rats are sensitive to an aversive taste quality in sucralose, whereas mice are not. This difference in taste between closely related rodents is worthy of further study.

Sucralose clearly does not taste like Splenda to rats. Most notably, aversion to sucralose was not on display in the Splenda tests. LoS and HiS rats preferred Splenda solutions to water, even though those solutions contained the same amount of sucralose as solutions rejected by half the rats in Experiment 3. In fact, in both tests at the highest Splenda concentration, not a single rat was averse to Splenda. For some rats, the "bulking" ingredient maltodextrin makes an otherwise unpalatable sucralose solution palatable.

Multi-saccharide or multi-sweetener solutions can be more palatable than constituent solutions in rats and humans (Smith and Foster 1980; Sclafani, Einberg, and Nissenbaum 1987; Hanger et al. 1996), and a high-potency sweetener can further increase intake of a sucrose–maltodextrin mixture (Sclafani et al. 1998). Sclafani and Clare (2004) reported that rats do not prefer a sucrose–sucralose mixture to sucrose alone but do tend to drink more of the sucrose + sucralose solution than plain sucralose tested separately. Whereas sucralose does not synergistically increase intake of

carbohydrate solution as saccharin does (Smith and Foster 1980; Sclafani et al. 1987; Sclafani and Clare 2004), sucrose can increase sucralose intake, either by masking its aversive side taste or by simply increasing drinking in an additive fashion. Tests with various sucralose–saccharide mixtures could establish the relative effectiveness of saccharides in functionally inhibiting sucralose's aversive properties. Furthermore, maltodextrin is a nutrient, and the 23-h tests used here could allow for nutrient-based flavor preference conditioning as a means through which preference for the taste of sucralose and/or the sucralose–maltodextrin mixture was enhanced (Ackroff and Sclafani 1994; Ramirez 1995; Delamater et al. 2006). Brief taste tests, flavor conditioning, and sham-drinking studies would be useful in determining the role of unconditioned sensory factors versus perceptual learning or postingestive feedback in preference for sucralose–saccharide mixtures.

HiS and LoS rats were distinguished more by amount of SC45647 or Splenda consumed than by how strongly they preferred those tastants to water and were distinguished more by aversion to sucralose than by consumption of it in excess of water baseline. Dissociations between avidity of consumption and preference/aversion are consistent with partially independent relationships of taste to "wanting" (incentive salience) versus "liking" (hedonic evaluation; Berridge 2004). Insofar as the wanting/liking distinction is relevant to the present results, the line difference in wanting might be more robust than liking for palatable tastants, and the converse for complex tastes with an aversive component. These possibilities could be evaluated using methods appropriate to distinguishing tastants' incentive and hedonic properties.

Whereas individual differences among rats in sucralose's taste are clear, individual differences among humans in the taste qualities of aqueous sucralose remain largely unexamined (see Kamerud and Delwiche 2007). Zhao and Tepper (2007) reported that 6-n-propylthiouracil (PROP) supertasters perceived orange soda sweetened with sucralose (0.163 g/L), corn syrup, or other sweeteners as more bitter than did PROP nontasters. The group difference was comparable for all sweeteners. The helpfulness of this finding for present purposes is limited by the use of a sour vehicle and a single sucralose concentration. Schiffman et al. (1995) showed that people's ratings of sucralose's bitterness increase in the same concentration range as was used in Experiments 2 and 3, and Frank et al. (2008) showed that sucralose activates brain regions associated with taste pleasantness less effectively than sucrose. However, whether individual differences in sucralose's taste are more or less marked than in rats, stable across concentrations, or covary with saccharin's bitterness is unknown.

Whether the difference in rats' response to sucralose and Splenda reported here would occur in humans also remains to be determined. Humans and other primates apparently perceive sucralose as sweeter and less bitter than do rats, and Polydose compares less favorably to sugar in humans

and other primates than in rats (Feigin et al. 1987; Laska et al. 2001). However, sucralose does appear to have a fundamentally dual, bitter–sweet taste in humans. Moreover, the degree of polymerization of the maltodextrin in Splenda might be lower than for Polycose, which likely would make it sweeter and more palatable than Polycose. If basic mechanisms of individual differences in sucralose's taste are shared by humans and rats, maltodextrin potentially contributes to the breadth of Splenda's appeal: maltodextrin might have little effect among people insensitive to sucralose's aversive side taste but increase Splenda's palatability among those who, like LoS rats, detect it. Taste–taste conditioning and nutrient-based flavor conditioning (Yeomans et al. 2008) are other means by which maltodextrin could enhance the palatability of sucralose solution. Studies along these lines would provide useful data on tastant interactions that are missing from the human literature and also could settle the question of whether sucralose's aversive side taste is an important or trivial issue outside of the laboratory.

The present results reinforce the value of studying tastants with diverse species and subpopulations and of examining data for stable differences among individuals. These results also caution against interpreting similarities between closely related species too readily in terms of conserved taste receptor mechanisms. Though saccharin preference and some of its noningestive correlates seem similar in mice and rats (see Dess 2000), different taste receptor genes are involved (Lu et al. 2005). The possibility remains that they share mechanisms at the brain stem level or above (Sclafani 2006; McCaughey 2007) due to homology, convergent evolution, or epigenesis. With respect to the limited evidence available on sucralose's basic taste qualities, rats detect an aversive component and, in this respect, to perceive it more as do fruit flies and humans (Schiffman et al. 1995; Gordesky-Gold et al. 2008) than do mice, a pattern that calls for explanation. Continued efforts at building a diverse behavioral database are an important complement to theoretical work on the phylogeny of sweet taste affinity and its ecologically and developmentally contingent expression.

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