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Examination of the perception of sweet- and bitter-like taste qualities in sucralose preferring and avoiding rats

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Abstract

Sucralose avoiding rats detect a bitter-like taste quality in concentrations of sucralose that are strongly preferred over water by sucralose preferring rats. Here, we investigated whether sucralose preferrers (SP) also detect a bitter-like quality in sucralose that may be masked by increased perception of sucralose's sweet-like quality. A microstructural analysis of sucralose intake revealed that, at concentrations they avoided in preference tests, sucralose avoiders (SA) consumed smaller and fewer bouts of sucralose than SP. Interestingly, the concentration-dependent increase in sucralose preference in SP was not associated with larger bouts or increased lick rate, two measures that are expected to increase with increasing perceived sweetness. This suggests that SP can detect an aversive quality in sucralose, but this perception of a presumably bitter-like quality may be masked by increased salience of a sweet-like quality that sustains high levels of intake in SP. Further evidence for increased sweet-taste perception in SP, relative to SA, was obtained in a second study in which SP consumed more of a palatable sweet-milk diet than SA. These are the first data to suggest that SP are not blind to the bitter-like quality in sucralose, and that there may be differences in sweet-taste perception between SP and SA.

Keywords

artificial sweetener; individual differences; rats; taste

Introduction

Although the role of variable taste perception in guiding diet choice has interested researchers for decades, it has proven difficult to study. Attempts to link genetic variation in human bitter-taste perception to feeding behavior and body mass index have produced mixed results [1–5]. While some studies were unable to link taste sensitivity to measures of food preference [6, 7], others strongly suggested that variation in the perception of taste

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quality may contribute to phenotypic variation in dietary preferences [8, 9]. These equivocal findings may be related to a number of factors, including a lack of uniformity in assessing taste sensitivity, heavy reliance on self-report measures of caloric intake, and the difficulty in controlling for cognitive factors, such as dietary restraint, which can modulate diet choice and caloric intake [10].

To address the relationship between taste perception and diet choice and to avoid these potentially confounding variables, our lab and others have begun to investigate how natural variation in the taste preferences of laboratory rats may affect food intake and dietary preferences [11–14]. These studies have shown that rats display considerable variation in their intake and acceptance of the artificial sweetener sucralose in a 24-h two-bottle preference testing paradigm, with ~70% of rats displaying either a modest preference or indifference for low concentrations (<0.025 g/L) of sucralose over water but strongly avoiding sucralose at higher concentrations (sucralose avoiders, SA). The remaining ~30% of rats display a strong preference for sucralose across a wide concentration range (sucralose preferrers, SP) [12].

This non-overlapping variation in the acceptance of sucralose appears to be consistent with psychophysical studies in humans and rodents indicating that the sweet-like taste quality of many artificial sweeteners, including sucralose, is offset in some individuals by the perception of an aversive taste quality [15] that appears to be mediated by the activation of bitter taste receptors (T2Rs) and/or the transient receptor potential vanilloid-1 (TRPV1) receptor [16]. For instance, the variable acceptance of saccharin in humans is associated with allelic variation in *Tas2R31* [17]. These data, together with SA's strong avoidance of sucralose solutions > 0.25 g/L (as opposed to indifference), even in brief-access paradigms, suggested to us that SA detect an aversive taste quality in sucralose that SP either do not detect or perhaps do not respond to.

As an initial step toward uncovering the aversive nature of sucralose, and to determine if taste was sufficient to distinguish SA from SP, we used an adaptation of the two-alternative, forced-choice psychophysical paradigm [18]. This paradigm allowed us to determine that the perceived taste quality of sucralose differs between SP and SA. Briefly, animals were trained to report (via an operant response in a gustometer) whether the taste of a given concentration of sucralose generalized to a prototypical sweet-like stimulus (sucrose) or a prototypical bitter-like stimulus (quinine). While SP reported a sweet-like taste quality at all concentrations of sucralose that were treated as different than water (i.e., assumed to be above threshold within this paradigm), SA were more likely to generalize the taste of these same concentration of sucralose to quinine [19]. These data provide clear evidence that SA detect a bitter-like taste quality in normally avoided sucralose concentrations. SP also licked more to sucralose than SA in a brief-access paradigm at these same concentrations [19]. Taken together, these findings confirm that differences in sensory (taste-guided) processing are sufficient to explain the differential acceptance of sucralose in SP and SA. They also confirm that SA detect an aversive taste quality in sucralose, but do not address whether or not SP are “taste-blind” to this component. This is because animals were forced to choose if the solutions being presented in the gustometer were either sucrose-like or quinine-like. Thus, in the case of a mixture, animals would be expected to choose the taste quality that is

more salient to them. Indeed, when the same animals were offered test solutions containing varying mixtures of sucrose and quinine, they reported a sweet-like quality in solutions containing low, suprathreshold concentrations of quinine, and did not report the presence of a bitter-like quality until quinine was sufficiently concentrated and presumably the more salient taste quality within the test solution [19]. Thus, while SP reported that the salient taste quality of sucralose was sweet-like, we cannot infer that “sweet” was the sole quality detected by SP or that SP were unable to perceive a bitter-like taste quality in the sucralose solutions. Rather, it merely suggests that SP’s perception of “bitter” did not surpass the salience of “sweet”.

Recent work from our lab provides clear evidence that the differences in taste perception between the groups are not unique to sucralose and that the differences in taste perception between SA and SP drive differences in their intakes of other binary mixtures such as saccharin and sucrose-base solutions adulterated with increasing concentrations of quinine [20]. However, to date our work has not addressed the degree to which these divergent phenotypes are mediated by perceptual differences in sweet and/or bitter taste.

It is essential to understand the nature of the perceptual differences between these animals as such information is prerequisite to identifying mechanisms that may be driving the differences in the taste-guided behavior and therefore allowing comparisons to variation in other populations. One possibility is that SA are sensitive to a bitter quality in sucralose that SP are less sensitive to, or, perhaps, insensitive to. This would suggest the underlying mechanism driving the phenotypic split may lie in bitter-taste signaling pathways, possibly at the receptor level, as is seen in human variation in the ability to taste 6-*n*-propylthiouracil (PROP) [21–23]. Some studies have shown that PROP tasters are more sensitive than PROP non-tasters to certain sweet and bitter foods, the bitter-like taste quality of saccharin, the creaminess of fats, and stimuli that cause oral burn [8, 24–27]. Another possibility is that SP are more sensitive to the sweet-like quality of sucralose than SA, and this increased perception of sweet taste may overshadow the perception of any bitter-like quality in sucralose. This would suggest the underlying mechanism driving the phenotypic difference is in the sweet-taste signaling pathways, as seen in mouse variation for sucrose avidity. A polymorphism in the gene encoding the T1R3 subunit of the sweet taste receptor in mice has been shown to contribute to between-strain variation in avidity for sucrose [13, 28]. At present, the degree to which either (or both) of these mechanisms mediates the phenotypic variation in SP and SA remains unclear.

As an initial step toward evaluating the taste-related perceptual differences in SP and SA, we conducted a microstructural analysis of sucralose drinking during a series of 24-h, 2-bottle preference tests used to categorize rats as SP or SA. Previous research has shown that the number and size of drinking bouts, and the rate of licking, can be used to make inferences regarding the palatability of a taste stimulus. For example, a microstructural analysis of saccharin drinking revealed decreases in bout size and the rate of licking as a function of increasing concentration, reflecting decreased palatability as the perception of a bitter-like quality increased [29]. A similar analysis of sucrose drinking revealed increases in bout size and the rate of licking as a function of increasing concentration, reflecting increased perception of sweetness [29]. Thus, this microstructural analysis was chosen for its ability to

assess sensitivity to both bitter- and sweet-like taste qualities in increasing concentrations of sucralose with a greater resolution than has been employed in other intake tests conducted to date [12, 20]. To better understand the functional consequences of the differences in taste perception in SP and SA, we conducted a second experiment to determine whether a heightened perception of “sweet” taste would promote greater intake of a palatable, sweetened-milk diet in SP, relative to SA.

Methods

Experiment 1a, Microstructural analysis of sucralose preference trials

Animals and housing—Male Long-Evans rats (n=24, Charles River Breeding Laboratory, Raleigh, NC), weighing 200–250 g at study onset, were individually housed in custom-designed Plexiglas cages. Food compartments at the front of the cages were equipped with infrared light-emitting diodes and photo detectors, which were used to monitor feeding bouts. The back of the cages held two drip-resistant bottles that were equipped with contact lickometers, which recorded individual licks and were used to monitor the size and duration of drinking bouts. Rats were allowed *ad libitum* access to Purina 5001 and tap water in addition to the test solutions. The colony room was maintained at a $20 \pm 2^\circ\text{C}$ with a 12:12 h light/dark cycle. All animal procedures were approved by the Florida State University Animal Care and Use Committee.

Experimental design—All rats were categorized as SP or SA via a series of 24-h two-bottle preference tests [12]. Rats were given water and increasing concentrations of sucralose (0.0001, 0.001, 0.01, 0.25, 0.5, 1.0, and 2.0 g/L) for two days per concentration with bottle position alternated daily. Rats with a side preference were excluded from the study (n=4). Sucralose solutions were prepared by dissolving various concentrations of sucralose (Tate & Lyle) in tap water. Rats were categorized as SP if they displayed a preference (consumed > 50% of daily fluid as sucralose) at the two highest concentrations; the remaining rats were categorized as SA. Because SA are more than twice as common as SP in the population [12], the data from all SP and an equal number of SA (selected for having the lowest preference scores at 2 g/L sucralose) were analyzed (n=8 per group).

Microstructure of licking was recorded throughout the process of categorizing rats as SP or SA. Drinking bouts of each test solution (i.e., water and sucralose) were defined by a minimum of 10 licks of the sipper tube of interest. Although two bottles were presented to the rat, a drinking bout was defined by licking activity at a single bottle and was not considered cumulative between the bottles. Drinking bouts were considered terminated when no licks were recorded for > 5 min on the bottle of interest. Rats regularly switched between bottles within individual bouts we analyzed these data in addition to the single bottle bouts. These “switches” were counted as any bout that contained, or was immediately followed (within 60 s of the last activity) by a bout from the alternate bottle [29].

Experiment 1b, Microstructural analysis, abbreviated concentration curve

Because sucralose intakes dropped precipitously from a high level (at concentrations 0.01 g/L) to near 0 (at concentrations 0.25 g/L) in SA, comparisons between groups in this first

cohort of rats were unable to elucidate the nature of the SA's initial avoidance to intermediate concentrations. As such, we repeated our microstructural analysis of sucralose intake in a new cohort of rats. This analysis was restricted to two new concentrations (0.05 and 0.1 g/L; intermediate to the concentrations between which we observed the precipitous drop in sucralose intake in SA), with the expectation that SA would display a more gradual decline in sucralose intake across these concentrations.

Animals and housing—Male Long-Evans rats (n=24), weighing 200–250 g at study onset, were categorized as SP or SA using the two-bottle preference testing procedure described above. All SP and an equal number of SA were selected for further testing (n=6 per group).

Experimental design—The microstructure of licking was recorded in SP and SA given access to water and two concentrations of sucralose (0.05 and 0.1 g/L, presented in ascending order) for two days per concentration as described above.

Experiment 2, Measure of sweetened milk consumption in SP and SA rats

Animals and housing—Female Long-Evans rats (n=20), weighing 230–260 g at study onset, were individually housed in custom-designed Plexiglas cages equipped with drip-resistant bottles and feeding niches that provided access to spill-resistant food cups. Because previous research has confirmed that the preference for sucralose, and the categorization of rats as SP or SA, is not sexually dimorphic [12], females were used in this study for two reasons. First, the goal of this study was to provide a palatable, sweetened diet (chow plus sweet milk as described below) that would increase daily food intake over that of chow alone, and previous research has shown that female rats overconsume this palatable diet to a greater extent than male rats [30]. Second, daily body weight gain is greater and more variable in male rats, relative to female rats [31], and we wanted to minimize any confounding influence of weight-related increases in energy intake during chronic access to the palatable diet.

Experimental design—Rats were categorized as SP or SA as described above, but using a more limited range of sucralose concentrations (0.001, 0.01, 0.1 and 1.0 g/L) as described in previous experiments [20]. All SP and an equal number of SA were selected for further testing (n=6 per group).

In the first phase of this experiment, rats were given free access to chow (Purina 5001), tap water, and palatable sweetened-condensed milk (Eagle Brand; diluted 1:2 with tap water; 68% carbohydrate, 21% fat, and 9% protein) for one week. The sweetened-condensed milk was chosen to provide a palatable diet in which sweet was likely the most salient quality (100% of the carbohydrate content in the sweet milk was in the form of sugars) to the rat's standard chow diet. Chow intake, milk intake and body weight were measured daily.

Following chronic access to the milk-supplemented chow diet, animals were maintained on chow and water alone for one month before assessing intake during a short-term (30-min) milk test. Rats were transferred to test cages that were identical in dimension to home cages but equipped with bottles containing a single recessed sipper tube. Rats were given daily 30-

min access to a bottle containing 0.25 M sucrose until they consumed 0.5 ml of sucrose from the bottle on 2 consecutive days, confirming that the animal could reliably locate and access the sipper tube. For 11 of the 12 animals this represented 2 days of training; 1 animal required 5 training days. Following training, rats were given 30-min access to sweet milk 3 h after light onset for 5 consecutive days and intakes were recorded daily.

Data analysis

During two-bottle preference testing (Experiment 1a) all SP, but only 3 of 8 SA displayed bouts of sucralose consumption across the entire range of concentrations. Likewise, all SA but only 5 of 8 SP displayed bouts of water consumption across the entire range of sucralose concentrations presented. In order to maximize the number of animals included in our microstructural analyses, intake data collected at the highest sucralose concentration (2 g/L) were excluded. This resulted in the inclusion of 8 SP and 6 SA for the microstructural analysis of sucralose intake and 7 SP and 8 SA in the microstructural analysis of water intake. The rate of licking was calculated as number of licks/bout length, to calculate the licks/second and is averaged across all bouts in 24 h. Because SA consumed so little sucralose at concentrations 0.25g/L we did not include the rate of licking for SA consuming sucralose.

Solution intakes and microstructural measures during two-bottle preference tests (Experiment 1a & b) were monitored daily and then averaged across the 2 test days of each sucralose concentration. Average preference for each sucralose concentration was calculated by dividing average sucralose intake by average total fluid (sucralose plus water) intake and expressing the scores as a percentage. Intakes, preferences and microstructural variables of sucralose and water licking were analyzed using a 2-way ANOVA with group (SP and SA) as the between-subjects variable and concentration of sucralose as the within-subjects variable. Tukey's honestly significant difference post hoc testing was used to explore significant main or interactive effects ($P < 0.05$).

In Experiment 2, chronic (one-week) milk intake was analyzed via a mixed-design repeated-measures ANOVA, with group (SP and SA) as the between-subjects variable and time (days) as the within-subjects variable. Average milk intakes during the final four days of 24-h testing and brief access (30-min) tests were analyzed with independent t-tests comparing intakes between SP and SA.

Results

Experiment 1a, Microstructural analysis of sucralose preference trials

Preference tests—As expected, preference for sucralose was influenced by a group by concentration interaction (Table 1, $F_{6,84} = 78.2$, $P < 0.001$). While SP and SA displayed a similar preference for sucralose at the 3 lowest concentrations, SP displayed a greater preference for sucralose than SA at the 4 highest concentrations (0.25–2.0 g/L, $P_s < 0.05$). SP displayed a concentration-dependent increase in sucralose preference that plateaued at 90–95% at 0.25 g/L sucralose ($P_s < 0.05$). In contrast, SA displayed a concentration-dependent decrease in sucralose preference that was characterized by a similar preference for the 3

lowest concentrations and then a pronounced avoidance at the 4 highest concentrations ($P_s < 0.05$).

Total fluid intake was also influenced by a group by concentration interaction ($F_{6,84} = 2.5$, $P < 0.01$). Although SP and SA consumed equivalent volumes of fluid when sucralose was presented at low concentrations, SP consumed more fluid than SA when sucralose was presented at concentrations > 0.25 g/L (Table 1, $P_s < 0.05$). This effect was driven by similar interactions for sucralose and water intakes ($F_{6,84} = 66.4$ and 52.6 , respectively, $P_s < 0.001$), with SP preferentially consuming sucralose and SA preferentially consuming water when the four highest sucralose concentrations were available (Table 1, $P_s < 0.05$).

Microstructural variables, sucralose only bouts—SP and SA differed in the amount of sucralose consumed per bout and the number of bouts as a function of sucralose concentration (Fig. 1a,b, $F_{5,60} = 25.6$ and 41.5 respectively, $P_s < 0.001$). While SP and SA displayed equivalent consummatory behaviors at concentrations < 0.25 g/L, SP drank more sucralose per bout and took more bouts than SA at concentrations > 0.25 g/L ($P_s < 0.05$). SP did not alter bout size as a function of concentration, but they did initiate more bouts as the concentration increased ($P_s < 0.05$). SA, in contrast, decreased bout size and initiated fewer bouts with increasing concentration ($P_s < 0.05$). Inter-bout interval (IBI; the time between bouts of sucralose) was influenced by a group $\times$ concentration interaction ($F_{5,60} = 16.5$, $P < 0.01$). Post-hoc analysis revealed that SA, but not SP, increased the time between bouts of sucralose as a function of concentration ($P < 0.05$; data not shown). Analysis of lick rate was limited to SP due to the dramatic decrease in bout size beyond that of our stated criterion in SA at the three highest sucralose concentrations. This analysis revealed that SP displayed a significant decrease in the rate of licking as a function of sucralose concentration (Fig. 1c, $F_{5,35} = 5.9$, $P < 0.001$), with the lowest lick rates observed at the three highest sucralose concentrations ($P_s < 0.05$).

Microstructural variables, water only bouts—SP and SA differed in the amount of water consumed per bout and the number of bouts consumed as a function of sucralose concentration (Table 2, $F_{5,65} = 6.0$ and 23.7 respectively, $P_s < 0.01$). Post-hoc testing revealed that bout size decreased as a function of concentration in SP ($P_s < 0.05$), but not SA. Bout number decreased in SP, and increased in SA, as a function of concentration ($P_s < 0.05$). IBI was also influenced by a significant interaction between concentration and sucralose preference ($F_{5,65} = 6.6$, $P < 0.001$). IBIs were longer while SP had access to high concentrations of sucralose than while SA had access to the same concentrations (data not shown). The rate of licking of water was affected by a group $\times$ concentration interaction ($F_{5,65} = 2.3$, $P = 0.05$), however, post-hoc tests revealed no differences between the groups or concentrations within a group.

Bout switches—To better understand how water and sucralose were consumed with respect to each other we investigated the percentage of licking bouts during which the rat consumed both fluids in a single drinking bout. Switching from sucralose to water and water to sucralose were both influenced by group $\times$ concentration interactions (Fig. 2a, $F_{5,60} = 15.7$, $P < 0.01$ and Fig. 2b, $F_{5,55} = 10.8$, $P < 0.01$). Although SP and SA switched between solutions an equivalent percentage of times at concentrations < 0.25 g/L, at concentrations

0.25 g/L SA switched to water during a greater percentage of sucralose bouts and SP switched to sucralose during a greater percentage of water bouts ($P_s < 0.05$).

Experiment 1b, Microstructural analysis, abbreviated concentration curve

Microstructural variables, sucralose—SA displayed lower preference for sucralose than SP independent of concentration (Fig. 3a, $F_{1,14} = 16.3$, $P < 0.01$). Although there was no effect of concentration in either group, (Fig. 3b, $F_{1,13} = 0.21$, $P = 0.65$), bout size was influenced by a group $\times$ concentration interaction (Fig. 3b, $F_{1,13} = 9.0$, $P = 0.01$), with SP consuming larger bouts of sucralose than SA at 0.1 g/L ($P < 0.05$), but not at 0.05 g/L. There was a main effect of group on bout number (Fig. 3c, $F_{1,13} = 13.6$, $P < 0.01$), with SP drinking more bouts of sucralose than SA at both concentrations ($P_s < 0.05$). There were also significant main effects of group ($F_{1,13} = 13.3$, $P < 0.01$) and concentration ($F_{1,13} = 9.6$, $P < 0.01$) on IBI, as well as a significant interaction ($F_{1,13} = 9.0$, $P = 0.01$). SP decreased IBI with increasing concentration whereas SA increased IBI with increasing concentration ($P_s < 0.05$). Finally, there was no effect of group on the rate of licking ($F_{1,13} = 1.8$, $P = 0.2$).

Bout switches—There was no effect of group or concentration on the percent of sucralose bouts containing a water bout but SA, relative to SP, tended to follow a higher percentage of sucralose bouts with a water bout as the concentration increased (Fig. 3e, $F_{1,11} = 4.3$, $P = 0.06$). SP were more likely than SA to follow a water bout with a sucralose bout (Fig. 3f, $F_{1,11} = 13.5$, $P < 0.01$) at both concentrations ($P_s < 0.05$).

Experiment 2, Sweet diet intake

Consumption of the milk diet varied across the 7-day intake test (Fig. 4a, $F_{6,60} = 8.75$, $P < 0.01$), but no main or interactive effects of preference status were detected (Fig. 4a, $F_{1,10} = 1.0$, $P = 0.3$, $F_{6,60} = 1.14$, $P = 0.3$). Post hoc tests revealed, however, that milk intake increased as a function of diet exposure in SP, but not SA ($P_s < 0.05$). This effect of sucralose preference status was confirmed by a second analysis of average milk intake during the last 4 days of the 7-day test, when daily milk intake was stable in both groups. This analysis revealed that average daily milk intake was increased in SP, relative to SA as measured by total intake (Fig. 4b, $t_{10} = 2.6$, $P = 0.03$) or as measured by ml/g body mass ($t_{10} = -2.14$, $P = 0.058$).

To investigate whether the daily overconsumption of milk intake in SP, relative to SA extended to a shorter-term intake test, milk was presented for 30-min per day for 5 days. Under these conditions, daily average milk intake did not differ in SP and SA (Fig. 4c, $t_{10} = 0.32$, $p = 0.76$).

Discussion

Our lab and others have demonstrated that rats display considerable variation in their acceptance of sucralose [12, 14, 32, 33], and that these differences are taste-driven [19]. However, the degree to which SP and SA differ in their perception of bitter- and sweet-like taste qualities remains unclear, as do the potential consequences of this differential perception of taste on caloric intake and diet choice. Here, we conducted a microstructural

analysis of sucralose and water intake during a series of two-bottle preference tests used to categorize rats as either SP or SA. In a second study, feeding behavior was monitored in SP and SA maintained on chow plus a palatable sweetened-milk diet for 7-days, as well as during a series of brief-access (30-min) sweetened-milk tests. Our findings confirm and extend previous reports that SA detect an aversive taste quality in sucralose, and provide the first evidence that sucralose is not perceived as a unitary sweet-like stimulus by SP. Furthermore, these data also provide the first evidence that the differential taste perceptions of SP and SA influence daily, but not brief-access, intake of a palatable sweetened-milk diet.

The microstructural analysis of licking behavior in Expt. 1a revealed that SA displayed a precipitous drop followed by a sustained decrease in both the size and number of bouts consumed at concentrations of sucralose that were profoundly avoided in preference tests (Fig. 1). This confirms previous reports that SA detect a concentration-dependent aversive taste quality in sucralose [12, 19, 20]. Our current study further revealed that SA switched from drinking sucralose to drinking water approximately 60% of the time at concentrations of sucralose that they avoid (Fig. 2). Thus, SA display a lack of motivation to drink sucralose to fluid repletion once licking is initiated. Because very little sucralose was consumed by SA at these higher concentrations, a follow-up study was conducted to provide a better depiction of the microstructural changes at concentrations intermediate to the concentrations which are preferred and rejected.

In this follow-up study, the decrease in sucralose intake in SA, relative to SP, was mediated initially by a selective decrease in the number of bouts consumed (at 0.05 g/L), and then by decreases in both the number and size of bouts consumed (at 0.1 g/L; Fig. 3). Available data suggest that these microstructural changes in licking behavior are mediated, at least in part, by the detection of a bitter-like taste quality. First, we have previously shown that SA generalize the aversive quality to quinine, a prototypical bitter-like stimulus [19]. Second, using the same testing paradigm as used here, Smith [29] has shown that a similar concentration-dependent decline in saccharin intake, an artificial sweetener known to activate T2Rs in both rodents and humans [17, 34, 35], is also mediated by a decrease in bout number followed by a secondary decrease in bout size. However, as some artificial sweeteners have been characterized as having a metallic taste [36], we cannot rule out the possibility that an aversive metallic-like taste may contribute to the aversive quality of sucralose.

The pattern by which SA initially decreased their intake of sucralose in Expt. 1b is interesting as it suggests that their motivation to consume 0.05 g/L sucralose was reduced, yet, they did not treat this concentration of sucralose as aversive within an ongoing drinking bout. Specifically, microstructural variables associated with palatability, such as bout size and rate of licking, did not differ between SP and SA at this concentration, implying that the reduction in daily intake and preference observed in SA was primarily driven by a decrease in their motivation to return to the bottle containing the sucralose solution. Moreover, there was no significant change in the rate of licking by SA at either of the two intermediate concentrations presented in Expt. 1b. These data extend our previous work by demonstrating that the initial emergence of SA's avoidance response (at 0.05 g/L sucralose) is mediated by a decrease in their motivation to consume sucralose rather than the detection of an aversive

taste quality during the ongoing ingestive bout. Thus, the decreased intake at this concentration is likely mediated by an aversive internal state or the generation of an aversive off-taste that develops during the interbout interval. The additional decrease in bout size at higher sucralose concentrations suggests that SA eventually do detect an aversive, presumably bitter, taste quality during ongoing bouts of consumption. Future studies could address whether or not there is a difference between SA's and SP's perception of the solution during active drinking bouts at these concentrations. These questions could be addressed, in part, by analyzing microstructural variables at an even finer scale (e.g. licking cluster size and number) within a single drinking bout.

Although SP preferred sucralose across all concentrations, and this preference increased as a function of concentration, the microstructural data suggest that SP did not perceive sucralose as a unitary sweet-like stimulus. Previous research has demonstrated that rats display a robust increase in both bout size and the rate of licking with increasing concentration of a purely sweet stimulus (i.e., sucrose) during sham feeding tests when postingestive effects are negligible [29]. Here, SP did not alter the size of their licking bouts as a function of increasing sucralose concentration, a pattern of results contrary to the assumption that SP perceive sucralose as a unitary sweet-like stimulus. Furthermore, in the current study, SP decreased their rate of licking as sucralose concentration increased. Decreases in lick rate have been reported for stimuli that elicit a concentration-dependent aversive taste quality such as concentrated saccharin solutions [29]. Taken together, our current findings suggest that SP did, in fact, detect an aversive taste quality in sucralose. Despite this, SP maintained a high preference for sucralose and increased the number of bouts as a function of increasing sucralose concentration suggesting that their perception of any aversive quality was not salient enough to decrease total intake. Likewise, SP did not decrease IBIs while consuming sucralose, suggesting that they remain motivated to consume the solution. As such, these microstructural data are the first to indicate that SP are not, in fact, "blind" to the aversive quality of sucralose (i.e. they reduce the rate of licking) but rather may be more sensitive to the palatable sucrose-like taste quality [19] elicited by sucralose (i.e. they find the solution more motivating).

While these data provide the first demonstration that SP can detect the aversive taste quality of sucralose, they do not confirm whether a heightened sweet perception, serving to attenuate the saliency of any bitter quality, is responsible for maintaining the high preference for and consumption of sucralose in SP, relative to SA. If SP and SA do differ in their perception of sweet-like compounds, one might expect SP to consume more of a sweetened solution than SA. However, we did not see any microstructural differences in how SP and SA consumed low, equally preferred, concentrations of sucralose. At these low concentrations (0.0001 – 0.01 g/L), lick rate and bout size were not increased in SP, relative to SA. While these data suggest that SP did not perceive an increase in the saliency of the sweet-like taste of sucralose at these concentrations, it should be noted that these concentrations are treated as similar to water in brief-access tests [19], so the sweet-like quality may not be salient enough at these concentrations to drive a difference between the groups as these three concentrations may approach detection thresholds in both groups of rats.

A final study was conducted to investigate “sweet”-driven intake in the absence of any potential aversive stimuli. We were interested in how differences in taste perception might drive caloric intake and hypothesized that an increase in the perceived palatability of sweet-tasting food would drive SP to consume more calories than SA. To investigate this hypothesis, we supplemented the rats’ chow with palatable sweetened-milk. While this highly palatable diet increased daily caloric intake in both groups, SP consumed more sweetened milk than SA following adaptation to the novel diet. Thus, under these test conditions, “sweet”-taste perception appeared to be increased in SP, relative to SA. In comparison, we found no evidence that SP over-consumed this same diet during brief-access (30-min) tests. These disparate findings may be related to the fact that intermittent access to palatable food is highly rewarding (see [37]), making it possible that the restricted access to milk in the 30-min study was sufficient to mask potential intake differences between SP and SA. It may also be the case that this effect is relatively small and thus requires longer periods of time to produce measurable differences in intake. An important difference between the two studies is that the long-term access test provided dietary choice (i.e. chow versus sweetened-milk), while the brief-access test was limited solely to milk intake. As such, the differences between SP and SA in response to sweet milk may not be entirely sensory based, but may encompass a number of postingestive factors. Furthermore, sweetened milk is 68% carbohydrate, 9% protein and 21% fat. Thus, it is possible that the fat or protein content of the milk diet may have contributed to the differential intakes recorded in the long-term study. We are currently conducting more detailed dietary manipulations to investigate this.

In summary, the current findings suggest that the behavioral differences observed in SP and SA may not be solely driven by a differential sensitivity to an aversive side-taste in sucralose, but could also be driven by differential sensitivity to the palatable taste of sucralose at supra-threshold concentrations. Additional support for the differences in the perception of sweet-like stimuli was obtained in our feeding study as SP consumed more of a palatable sweetened-milk diet than SA when it was offered in addition to the animal’s standard diet of rat chow. While the current findings do not definitively distinguish between the specific contributions of altered “bitter” versus altered “sweet” taste perception in driving the differential intakes of sucralose and other binary solutions in SP and SA, they do provide converging support for the notion that SP may be more motivated by the reinforcing properties of the “sweet” tasting solutions than SA, and as such represent an important initial step towards identifying the mechanism underlying this phenotypic split in acceptance of sucralose. Our work is the first to suggest that SP detect a bitter-like taste in sucralose. Thus, there is a need for future studies to determine if there are differences between the detection thresholds for ‘sweet’ and ‘bitter’ stimuli in SA and SP. Furthermore, the natural variation in the acceptance of sucralose provides a promising model to study, more broadly, the impact of variable taste perception on ingestive behavior.

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Highlights

- Sucralose avoiding rats detect an aversive taste quality in sucralose.
- Sucralose preferring rats do not treat sucralose as a unitary sweet stimulus.
- Sucralose preferring rats consume more sweet milk diet than sucralose avoiding rats.

Figure 1.

Figure 1 represents licking microstructure analyses in SP and SA. Data are the mean ($\pm$ standard error of the mean). A: SA and SP consumed equivalent bouts of sucralose at low concentrations (< 0.25 g/L) but SA consumed minimal bouts of sucralose at concentrations ≥ 0.25 g/L. B: Bout number was similar between SA and SP at low concentrations (< 0.25 g/L) but SA drank very few bouts at concentrations ≥ 0.25 g/L. C: SP decrease the rate of licking as a function of increasing concentration. SP (n=8), SA (n=6). Different letters denote within-group differences while (*) denote between-group differences (P<0.05).

Figure 2.

Figure 2 represents the percentage of fluid bouts that contain a fluid bout from the opposing bottle. Data are the mean ($\pm$ standard error of the mean). A: At concentrations ≥ 0.25 g/L SA followed a higher percentage of sucralose bouts with water bouts relative to SP. SP (n=8), SA (n=6) B: At concentrations ≥ 0.25 g/L SP followed a higher percentage of sucralose bouts with water bouts relative to SA. SP (n=7), SA (n=8). Different letters denote within-group differences while (*) denote between-group differences ($P < 0.05$).

Figure 3.

Figure 3 represents licking microstructure analyses in SP and SA. Data are the mean ($\pm$ standard error of the mean). A: SP show a greater preference for sucralose over water at both concentrations. Preference = sucralose intake/ total fluid intake, expressed as percentages. SP (n=8), SA (n=8) B: SP and SA consumed equivalent bouts of sucralose at the 0.05 g/L concentration but SA reduced bout size at the higher concentration. SP (n=8), SA (n=7) C: SP consume more bouts of sucralose at both concentrations. SP (n=8), SA (n=7) D: SP and SA did not differ in the rate of licking between groups or across concentration. SP (n=8), SA

(n=7) E: SP and SA did not significantly differ in the percentage of sucralose bouts that were followed by water bouts. SP (n=8), SA (n=8) F: At concentrations 0.25g/L SP follows a higher percentage of water bouts with sucralose. SP (n=8), SA (n=8). Different letters denote within-group differences while (*) denote between group differences ($P<0.05$).

Figure 4.

Figure 4 represents milk intake in SP and SA, data are the mean ($\pm$ standard error of the mean). A: Although overall SP and SA did not differ across the 7 day exposure, SP increased intake with increasing days of exposure. A similar effect was not observed in SA. B: SP consumed more milk on average during the final four days of exposure than SA, once intake was stable. B: SP and SA consumed equivalent amounts of milk in 30 min. SP (n=6), SA (n=6). Different letters denote within-group differences while (*) denote between-group differences ($P < 0.05$).

Table 1

Data are presented as means $\pm$ standard error of the mean. Rats were given a series of 2-bottle preference tests involving water versus an ascending series of sucralose concentrations. Rats were categorized as SP if they displayed a preference (consumed $> 50\%$ of their daily fluid as sucralose) at the two highest concentrations; the remaining rats were categorized as SA. Intakes and preferences were analyzed using a 2-way ANOVA with group (SP and SA) as the between-subjects variable and concentration of sucralose as the within-subjects variable. Tukey's honestly significant difference post hoc testing was used to explore significant main or interactive effects.

		Concentration of sucralose (g/L)							
		(n)	0.0001	0.001	0.01	0.25	0.5	1.0	2
% preference	SA	8	52.9 $\pm$ 5.4a	69.7 $\pm$ 6.9a	74.8 $\pm$ 8.2a	3.2 $\pm$ 0.6b*	2.9 $\pm$ 0.3b*	3.6 $\pm$ 0.3b*	2.5 $\pm$ 0.2b*
	SP	8	64.5 $\pm$ 5.6a	65.5 $\pm$ 6.3a	75.9 $\pm$ 6.4ab	87.7 $\pm$ 4.0b	95.9 $\pm$ 1.5b	92.7 $\pm$ 2.6b	93.3 $\pm$ 1.8b
total fluid intake	SA	8	49.1 $\pm$ 2.3	48.2 $\pm$ 2.0	49.3 $\pm$ 5.9	47.0 $\pm$ 1.9*	48.3 $\pm$ 2.0*	48.2 $\pm$ 1.8*	47.8 $\pm$ 1.5*
	SP	8	49.1 $\pm$ 2.6a	49.4 $\pm$ 2.6a	50.9 $\pm$ 2.9ab	59.4 $\pm$ 2.6b	59.6 $\pm$ 2.6b	58.6 $\pm$ 2.4ab	58.5 $\pm$ 3.1ab
water intake	SA	8	22.8 $\pm$ 2.8a	14.2 $\pm$ 3.0a	11.2 $\pm$ 4.5ab	45.5 $\pm$ 1.9b*	46.9 $\pm$ 1.9b*	46.4 $\pm$ 1.8b*	46.6 $\pm$ 1.4b*
	SP	8	17.6 $\pm$ 3.1a	17.6 $\pm$ 3.7a	12.9 $\pm$ 4.2ab	7.7 $\pm$ 2.7b	2.6 $\pm$ 1.1b	4.4 $\pm$ 1.8b	4.1 $\pm$ 1.2b
sucralose intake	SA	8	26.2 $\pm$ 2.3a	34.0 $\pm$ 4.0ab	37.5 $\pm$ 5.6b	1.5 $\pm$ 0.3c*	1.4 $\pm$ 0.1c*	1.7 $\pm$ 0.2c*	1.2 $\pm$ 0.1c*
	SP	8	31.5 $\pm$ 3.3a	31.8 $\pm$ 2.7a	37.9 $\pm$ 2.9ab	51.7 $\pm$ 2.1a	57.0 $\pm$ 2.0b	54.2 $\pm$ 1.3b	54.5 $\pm$ 2.3b

Different letters denote with-in group differences while (*) denote between-group differences ($P < 0.05$).

Table 2

Data are presented as means $\pm$ standard error of the mean. The table represents licking microstructure analyses of water in SP and SA. Water was presented in a 2-bottle test along with increasing concentrations of sucralose. Microstructural variables were analyzed using a 2-way ANOVA with group (SP and SA) as the between-subjects variable and concentration of sucralose as the within-subjects variable. Tukey's honestly significant difference post hoc testing was used to explore significant main or interactive effects.

			Concentration of sucralose (g/L)						
		(n)	0.0001	0.001	0.01	0.25	0.5	1.0	
bout size (licks/bout)	SA	8	278.1 ± 56.1ab	301.4 ± 33.5ab	228.6 ± 46.6a	403.3 ± 41.1b*	387.8 ± 37ab*	379.3 ± 37.1ab*	
	SP	7	260.3 ± 62.6ab	300.8 ± 67.5a	250.6 ± 58.7ab	91.2 ± 28.7b	88.7 ± 47.0b	133.9 ± 64.8b	
number of bouts	SA	8	9.5 ± 2.7a	9.0 ± 2.7a	7.2 ± 2.4a	21.4 ± 1.3b*	21.6 ± 2.0b*	22.4 ± 2.3b*	
	SP	7	12.3 ± 1.6ab	15.9 ± 2.9a	9.4 ± 2.0bc	7.7 ± 2.7bc	2.8 ± 0.6c	4.0 ± 1.3c	
rate of licking (licks/sec)	SA	8	0.9 ± 0.09	1.3 ± 0.17	1.2 ± 0.14	0.7 ± 0.17	1.2 ± 0.4	1.5 ± 0.5	
	SP	7	1.2 ± 0.12	1.2 ± 0.17	0.9 ± 0.15	0.8 ± 0.11	0.8 ± 0.11	0.7 ± 0.10	

Different letters denote with-in group differences while (*) denote between-group differences ($P < 0.05$).