Experience with Sugar Modifies Behavioral but not Taste-Evoked Medullary Responses to Sweeteners in Mice

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Abstract

Dietary exposure to sugars increases the preference for and intake of sugar solutions in mice. We used brief-access lick tests and multiunit electrophysiological recordings from the nucleus of the solitary tract (NST) to investigate the role of taste in diet-induced changes in sucrose responsiveness. We exposed C57BL/6J (B6) and 129X1/SvJ (129) mice to either a sucrose diet (chow, water, and a 500 mM sucrose solution) or a control diet (chow and water) for 3 days. In B6 mice, exposure to the sucrose diet decreased the appetitive response (i.e., number of trials initiated) but had no effect on the consummatory response (i.e., rate of licking) to 500 mM sucrose and 20 mM saccharin. In 129 mice, exposure to the sucrose diet increased the appetitive response but had no effect on the consummatory response to the sweetener solutions. In the NST recordings, the B6 mice exhibited larger multiunit responses to sweeteners than 129 mice, but there was no effect of the sucrose diet in either strain. Our results indicate that sucrose exposure alters the appetitive response of B6 and 129 mice to sweeteners in diametrically opposed ways and that these changes are mediated by structures in the gustatory neuraxis above the NST (e.g., ventral forebrain).

Key words: diet-induced plasticity, mouse, sucrose, sweet taste

Introduction

The factors that determine ingestive responses to sapid solutions are complex, with genetics and dietary experience playing major roles. For example, mouse strains vary widely in their preference for (and intake of) dilute concentrations of sweeteners during 24-h preference tests, resulting in the assignment of labels such as sweet “taster” and “nontaster” (Lush 1989; Capeless and Whitney 1995) or “sensitive” and “subsensitive” (Sclafani 2007) strains. Molecular biological studies revealed that sensitive and subsensitive mouse strains express different alleles of Tas1r3 (Kitagawa et al. 2001; Montmayeur et al. 2001; Sainz et al. 2001; Reed et al. 2004) and that the T1r3 protein in sensitive strains has a higher binding affinity for sweeteners (Nie et al. 2005). Additional studies established that the sensitive strains exhibit stronger consummatory responses (i.e., taste-mediated licking) for low but not high concentrations of sweeteners (Dotson and Spector 2004; Glendinning et al. 2005) and larger taste-evoked responses to sweeteners in both the chorda tympani (CT) nerve and the nucleus of the solitary tract (NST), which is first relay for taste processing in the medulla (Frank and Blizard 1999; Inoue et al. 2001; McCaughey 2007).

Dietary experience with sugars can override the influence of Tas1r3 polymorphisms on preferences for and intake of sugars. For example, sensitive (C57BL/6; henceforth, B6) and subsensitive (129P3/J and 129X1; henceforth, 129) mouse strains were offered a range of sweetener concentrations across a series of 23-h sweetener versus water tests. During the first test series, the 129 mice exhibited weaker preferences for and lower intakes of dilute concentrations of sucrose than did the B6 mice (Sclafani 2006b, 2007). However, when mice from both strains were retested with the same test series, they exhibited equally strong (>90%) preferences and attenuated differences in sucrose intake. There are additional reports of dietary experience with glucose, fructose, and galactose enhancing subsequent intake of the same or related chemical stimuli in B6 mice during 23-h preference tests (Zukerman et al. 2013).
limitation of these tests, however, is that their long duration makes it difficult to draw clear inferences about the mechanisms underlying the experiential effect. For example, we do not know whether the exposure regime actually increased the attractiveness of the sweeteners (e.g., made them taste better). Furthermore, there are multiple locations along the gustatory neuraxis, both peripheral and central, where dietary experience could exert its effects.

Here, we asked whether 3 days of exposure to a sucrose solution would increase the taste-mediated response to suprathreshold concentrations of sucrose and saccharin in B6 and 129 mice. In Experiment 1, we used a brief-access taste test (Glendinning et al. 2002) to assess sweetener acceptability. To this end, we measured the initial appetitive and consummatory responses to the sweeteners. For the appetitive response, we determined how many trials the mice initiated across a 30-min test session; and for the consummatory response, we determined the rate of licking once the sweetener solution had contacted the oral cavity. In Experiment 2, we made multiunit recordings from the NST during lingual stimulation with sapid stimuli. The NST was chosen because it receives gustatory input from all of the taste nerves; multiunit recordings were used because they reflect the contribution of a broad population of NST cells, including those that might be difficult to isolate from background noise owing to their small size. Furthermore, prior work suggests that the size of taste-evoked multiunit NST responses to sugars correlates well with their perceived intensity in rats (Giza and Scott 1987). Given that naive B6 mice are known to have both higher preferences for and larger taste-evoked NST responses to dilute sweeteners (compared with naive 129 mice), we asked whether strain differences in the latter measure are also attenuated by 3 days of sucrose exposure. Such an outcome would indicate that diet-induced changes in the early stages of taste processing (i.e., in the periphery or NST itself) mediated the previously observed plasticity in sweetener preference.

**Materials and methods**

**Animals and housing conditions**

We used 2 strains of mice, C57BL/6J (B6) and 129X1/SvJ (129), purchased from Jackson Laboratories (Bar Harbor, ME). For each strain and exposure condition, we used 9–11 mice in Experiment 1 and 6–7 mice in Experiment 2; we tested approximately equal numbers of males and females. The age range in Experiment 1 was 8–10 weeks and in Experiment 2 was 9–36 weeks. All mice were housed individually in standard polycarbonate tub cages (27.5 × 17 × 12.5 cm). The housing facility had automatically controlled temperature and lighting (12:12 h light:dark cycle). Except where noted otherwise, the mice were maintained ad libitum on TestDiet laboratory chow (Rat Diet 5012; PMI Nutrition) and tap water and were tested during the light phase of their light–dark cycle. In both experiments, mice from each strain and gender were assigned randomly to the experimental treatments. Experimental protocols were approved by the Institutional Animal Care and Use Committees at Columbia University (Experiment 1) and Ball State University (Experiment 2) and were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

**Experiment 1: Does dietary exposure to sucrose alter the acceptability of sweeteners?**

**Lickometer testing**

We used the brief-access taste test described in Glendinning et al. (2002). Each 30-min lick test was conducted in a gustometer (Davis MS160-Mouse; DiLog Instruments), which provided a mouse with access to a single sipper tube across successive 5-s trials, each separated by 7.5-s intertrial intervals. Each 5-s trial started after the first lick. To minimize the potential contribution of olfactory cues from the taste stimuli to the licking response, we passed a stream of air over the sipper tube (via a small fan) during each trial.

Prior to testing, each mouse received 3 days of training to familiarize it with the gustometer and train it to obtain fluid from the sipper tube. To motivate sampling from the sipper tube, the mouse was water-deprived for 22.5 h prior to the training session. Each training session began when the mouse took its first lick and lasted 30 min. On Training Day 1, the mouse could drink water freely from a single stationary spout throughout the training session. Immediately after this training session, the mouse was given 1 h of ad libitum access to water. Then, it was water-deprived for another 22.5 h. On Training Days 2 and 3, the mouse received water during successive 5-s trials. All mice adapted readily to the gustometer and took between 250 and 500 licks per training session. Following training, each mouse was given food and water ad libitum for 24 h.

We prepared the sweetener solutions immediately prior to testing. The sodium saccharin and sucrose (Sigma-Aldrich) were dissolved in deionized water and presented at room temperature. We selected 500 mM sucrose and 20 mM saccharin because these concentrations elicits strong appetitive licking in both mouse strains (Glendinning et al. 2005; Sclafani 2006a; Inoue et al. 2007). We included the saccharin solution to determine whether the effect of sucrose exposure would generalize to another sweetener.

Once training was completed, testing began. First, we subjected the mice to preexposure lick tests with the sucrose and saccharin solutions. Then, we exposed the mice to a sucrose or control diet (see below for details) over the next 3 days. Finally, we ran postexposure lick tests, with the same sweetener solutions. During each 30-min lick test, the mice were tested with a single sweetener solution and could initiate up to 144 trials. The gustometer recorded 1) the number of licks...
taken during each trial and 2) the number of trials initiated. The order of testing the 2 sweetener solutions (during both the pre- and postexposure lick tests) was counterbalanced across days to control for any potential order effects.

To encourage sampling from the sweetener solutions during the lick tests, we food and water restricted the mice for 23.5h immediately prior to the test. To this end, we placed each mouse in a clean cage with fresh bedding and provided it with 1 g of laboratory chow (dustless precision 1-g food pellets; BioServ) and 2 mL of tap water. Following the lick test, each mouse was given a recovery day, during which it had food and water ad libitum.

During the 3-day exposure period, the mice received ad lib access to chow, water, and 500mM sucrose (henceforth, sucrose diet) or chow and water (henceforth, control diet). Each mouse was housed individually in its home cage with chow and 2 bottles. For the control diet, both bottles contained water. For the sucrose diet, 1 bottle contained water, and the other contained 500mM sucrose. The mice accessed the solutions through sipper tubes located in the cage lid. The sipper tubes protruded approximately 1 cm into the cage, were positioned approximately 5 cm apart, and had a 1.5-mm hole designed for mice (Ancare). To control for position preferences, we alternated the left–right position of the bottles each day.

We measured daily chow and intakes from both bottles, as described elsewhere (Glendinning et al. 2010).

**Data analysis**

For the lick tests, we analyzed 2 dependent measures: number of trials initiated and standardized lick ratio (SLR). SLR is a measure of lick rate that controls for individual and strain differences in local lick rate. To calculate the SLR, we divided the mean number of licks emitted per trial for a given stimulus by the maximum number of licks that the same mouse could potentially emit across a 5-s trial. This latter value was calculated by multiplying each mouse’s local lick rate times 5 (i.e., the number of seconds in the trial). An SLR approaching 0.0 indicates that the taste stimulus elicited minimal licking, whereas an SLR approaching 1.0 indicates that the taste stimulus elicited maximal licking.

To calculate a mouse’s local lick rate (in licks/seconds), we used its licking responses during Training Day 1, when it had unlimited access to water. The first step involved deriving the mean interlick interval (ILI), which is defined as the average duration between the onsets of 2 consecutive licks. We limited our analysis to ILIs less than 200 ms because longer values are thought to reflect pauses between bursts of licking (e.g., see Spector et al. 1998). Then, we took the reciprocal of the mean ILI to determine the local lick rate.

For the lick data, we used SPSS Statistics to analyze the 2 lick measures (i.e., SLR and number of trials initiated) and each sweetener (i.e., sucrose and saccharin) separately. We ran mixed-model analyses of variance (ANOVAs), treating strain, sex, and exposure diet as between factors, and experience (i.e., pre- vs. postexposure responses) as a within factor. In this and all subsequent tests, significant main effects or interactions were followed by post hoc t-tests; we used an alpha level of 0.05.

To examine intake across the 3-day exposure period, we examined both daily sucrose solution intake and daily caloric intake (based on combined intakes of chow and sucrose solutions). The latter measure was estimated by multiplying daily intake (in gram) of laboratory chow × 3.43 kcal/g (provided by manufacturer) and daily intake (in gram) of 500mM sucrose solution × 0.685 kcal/g. We compared daily sucrose intake (or caloric intake) with 3-way mixed-model ANOVAs, treating strain, sex, and exposure diet as between factors, and exposure day as a within factor.

**Experiment 2: Does dietary exposure to sucrose alter responsiveness of the NST to sapid stimuli?**

Prior to making the neural recordings, each mouse was exposed to the sucrose or control diet for 3 days (see above for details). Intake of all fluids (i.e., 500mM sucrose and/or water) was measured using procedures described above.

**Neural recording**

Immediately following the exposure period, we measured taste-evoked multiunit activity in the NST. Methods were similar to those used previously for single-unit NST recording (McCaughey 2008), with the exception of using a larger electrode tip to avoid isolation of single units from background activity. Animals were anesthetized with a mixture of ketamine, xylazine, and acepromazine (90, 20, and 3 mg/kg, respectively, i.p., with additional doses as necessary). A tracheotomy was performed to prevent suffocation, and a fistula was inserted into the esophagus to prevent ingestion of taste solutions. The head was secured in a nontraumatic head holder modeled after that used in rats to avoid injury to the CT nerve. A section of skull overlying the cerebellum was removed, and the cerebellum was then aspirated to expose the surface of the medulla. Multiunit neural activity was isolated using glass microelectrodes filled with 1.6 M potassium citrate; the tip diameter was 5–10 μM, which is larger than that used for isolating single-cell activity. The signal was amplified, filtered, displayed on an oscilloscope, integrated with a time constant of 1 s, and recorded for off-line analysis using a 2-kHz sampling rate. Following identification of a taste-responsive location, 4 steps were taken before recording responses to taste stimuli: 1) the electrode was lowered and raised again to confirm that no gustatory single units stood out from the background neural activity anywhere within that vertical track; 2) there had to be a robust response to the NH₄Cl reference stimulus, including an initial phasic burst and a more sustained tonic component; 3) there needed to be minimal touch component to the neural activity, as gauged by only very small responses to water rinse or weak chemical stimulation; and
4) the baseline integrated voltage level and responsiveness to the reference stimulus had to remain fairly constant across a period of 5–10 min. The last 3 criteria were monitored throughout application of stimuli. If they were not met at any time, then the attempt was abandoned and another try was made at a different location. After successful application of all taste stimuli, the electrode position relative to obex was determined to confirm placement of the electrode in the NST. The mean coordinates across all animals that yielded usable recordings were 680 μM ventral to the surface of the medulla, 1.1 mm lateral to the midline, and 1.4 mm anterior to obex, which is consistent with a recording location in the rostral NST of mice.

Presentation of taste stimuli

Responses to 17 taste stimuli were measured: sucrose (10, 100, 500, and 1000 mM); sodium saccharin (1, 10, 20, and 50 mM); 500 mM glucose; 500 mM maltose; 100 mM glycine; 100 mM d-phenylalanine; 30% Polycose; 100 mM NaCl; 10 mM HCl; 20 mM quinine HCl; and 10 mM inosine monophosphate (IMP). In addition, 100 mM NH₄Cl was applied regularly (typically after every 4–5 nonammonium stimuli) to provide a reference stimulus. All chemicals, except Polycose, were purchased from Sigma-Aldrich; the Polycose was purchased from Abbott Laboratories. This large stimulus array permitted us to evaluate responses to sucrose and saccharin, nonsweet compounds, and several other sweeteners and carbohydrates that are thought to differ from sucrose in several respects (e.g., presence of nonsweet side tastes). We used NH₄Cl as a reference stimulus because 1) it is commonly used as a reference stimulus for multunit taste recordings; 2) there is no reason to expect its properties to be affected by sugar exposure; and 3) mean responses to NH₄Cl did not differ between B6 and 129 mice in single-unit NST recordings (McCaughey 2007).

All stimuli were dissolved in deionized water. Two milliliters of each stimulus was presented at room temperature at a rate of 1 mL/s, and it was rinsed off with at least 10 mL of deionized water. Stimuli and water rinse were delivered as a spray throughout the entire oral cavity. The concentration series of sucrose and saccharin were typically given in ascending order to minimize adaptation effects, and stimuli that are thought to share taste qualities (e.g., sucrose and glucose) were not given consecutively for the same reason. When possible, stimuli were presented multiple times and an average of all presentations was using in the data analysis. We also calculated the correlation between the relative response sizes of the first and second applications of a given stimulus for all instances in which they occurred in the same mouse. The result was +0.87, which suggests that the stability of our recording preparations was high and comparable with that found previously for single-unit NST recording in mice (McCaughey 2007).

Data analyses

We calculated the total integrated response (across 10 s) to each stimulus, based on the area-under-the-curve of the integrated voltage for 10 s after stimulus onset (evoked response) minus the area for 10 s before onset (baseline response). Relative responses to each stimulus were calculated by dividing the total integrated response to the taste stimulus by the total integrated response to the reference stimulus (i.e., 0.1 M NH₄Cl). Separate mixed-model ANOVAs were performed to examine the effects of strain, sex, exposure diet (between effects), and concentration (within effect) on relative responses to each of the sucrose or saccharin concentration series. A mixed-model 4-way ANOVA was performed to examine relative responses to each of the remaining 9 stimuli; we treated strain, sex, and exposure diet as between factors, and stimulus as a within factor. Significant main effects or interactions were followed by post hoc t-tests in order to look at differences for individual concentrations or stimuli.

We also examined the temporal properties of the multunit neural responses in more detail. Accordingly, we repeated the analyses described above but focused on the phasic (i.e., initial peak) or tonic (i.e., sustained) components of each taste response. The phasic response was defined as the maximum elevation of the integrated voltage, relative to the mean baseline level, within 3 s of stimulus onset, whereas the tonic response was the amount of voltage elevation at 10 s after onset. We found some minor differences between the phasic, tonic, and total integrated comparisons, but overall, the 3 measures yielded similar outcomes, and there were no unique insights provided by the phasic or tonic components of responses. We therefore present only total integrated responses below as they were more reliable and consistent.

Finally, to confirm that daily sucrose solution intakes across the 3-day exposure period during Experiment 1 were similar to those during the present experiment, we ran a 4-way mixed-model ANOVA, treating strain, experiment, and sex as between factors, and exposure day as a within factor. For this latter analysis, we focused on whether there was a significant main effect of experiment.

Results

Experiment 1: Does dietary exposure to sucrose alter appetitive or consummatory responses to sweeteners?

In Table 1, we show mean daily intake of the 3 dietary components plus total calories across the 3-day exposure period. For total caloric intake, there were significant main effects of diet (F1,32 = 8.9, P < 0.006) and day (F2,64 = 4.9, P < 0.02) but not of sex (F1,32 = 3.1, P > 0.08) or strain (F1,32 = 0.3, P > 0.6). There were no significant higher order interactions (in all cases, P > 0.05). It follows that mice from both strains consumed more calories/day on the sucrose than on the control diet. We also analyzed total intake of the 500 mM sucrose solution by mice on the sucrose diet. However, there were no significant effects
of day ($F_{1,32} < 0.1, P > 0.9$), sex ($F_{1,32} = 0.3, P = 0.6$), strain ($F_{1,32} = 0.4, P = 0.54$), or interaction of day × strain ($F_{2,32} = 0.3, P = 0.76$). Thus, sucrose intake (and hence, exposure) did not differ significantly across the strains.

In Figure 1, we show how dietary exposure to the control or sucrose diet modified the appetitive (i.e., number of trials initiated) and consummatory (i.e., SLR) phases of the ingestive response to 500mM sucrose and 20mM saccharin in B6 and 129 mice. With respect to the number of trials initiated for 500mM sucrose, there was a significant main effect of strain ($F_{1,32} = 8.9, P < 0.006$), but not of sex ($F_{1,32} = 0.5, P > 0.4$), exposure diet ($F_{1,32} = 2.1, P > 0.1$), or dietary experience (i.e., difference between pre- and postexposure responses) ($F_{1,32} = 0.1, P > 0.7$). There were significant interactions of strain × exposure diet ($F_{1,32} = 9.6, P < 0.005$) and strain × exposure diet × dietary experience ($F_{1,32} = 5.8, P < 0.03$). Post hoc tests revealed that dietary exposure to the sucrose diet (but not the control diet) altered the appetitive response (i.e., number of trials initiated) of both strains, but in different ways. A comparison of the number of trials initiated before and after exposure revealed that the B6 mice initiated significantly fewer trials (paired $t$-value = 2.5, degrees of freedom [df] = 10, $P < 0.04$) and the 129 mice initiated significantly more trials (paired $t$-value = 2.8, df = 8, $P < 0.03$) after exposure.

With respect to the number of trials initiated for 20mM saccharin, there were no significant main effects of strain ($F_{1,32} = 1.1, P > 0.25$), sex ($F_{1,32} < 0.1, P > 0.9$), exposure diet ($F_{1,32} = 0.2, P > 0.1$), or dietary experience ($F_{1,32} = 0.4, P > 0.5$). However, there were significant interactions of strain × dietary experience ($F_{1,32} = 5.2, P < 0.03$) and strain × exposure diet × dietary experience ($F_{1,32} = 5.8, P < 0.025$). Post hoc tests revealed that exposure to the sucrose diet (but not the control diet) changed the number of trials initiated by both strains. Indeed, the number of trials initiated by the B6 mice decreased with experience (paired $t$-value = 4.1, df = 10, $P < 0.003$), whereas the number initiated by the 129 mice increased (paired $t$-value = 2.5, df = 8, $P < 0.04$).

There were no apparent effects of diet, sex, or dietary experience on the SLR for 500mM sucrose and 20mM saccharin (in all cases, $P > 0.05$). There was a significant main effect of strain on both sweeteners (in both cases, $P < 0.006$), illustrating how the B6 mice licked more rapidly than did the B6 mice for 500mM sucrose and 20mM saccharin. There were no significant higher order interactions (in all cases, $P > 0.05$).

**Experiment 2: Does dietary exposure to sucrose alter responsiveness of the NST to sapid stimuli?**

Mean daily intake (±S.E.) of the 500mM sucrose by the B6 and 129 mice on the sucrose diet was 15.2g (± 0.8) and 13.8g (± 2.8), respectively. A comparison of daily sucrose solution intake by mice in this and the previous experiment (Table 1) revealed no significant main effects of experiment, strain, sex, or exposure day or higher order interactions (in all cases, $P > 0.05$). This establishes that mice in both experiments consumed statistically equivalent amounts of 500mM sucrose across the exposure period.

Figure 2 shows typical multiunit NST responses of mice from each treatment group, and Figure 3 shows mean response sizes to all stimuli. For the sucrose and saccharin concentration series, there were significant main effects of strain ($F_{1,17} > 10.1, P < 0.005$ in both cases) and concentration ($F_{4,51} > 35.4, P < 0.001$ in both cases), and a significant interaction of strain × concentration ($F_{4,51} > 7.4, P < 0.001$ in both cases). Post hoc tests revealed that the strain differences (i.e., B6 > 129) were significant at the 3 higher concentrations of the sweeteners ($P < 0.004$ in all cases) but absent at the lowest concentration of each compound. The main effects of preexposure diet and sex on the relative NST responses, and the other interaction terms including them, however, were all nonsignificant ($P > 0.05$ in all cases). In a comparison across the other 9 stimuli, there was a significant main effect of stimulus type ($F_{4,136} = 40.5, P < 0.001$) and a significant stimulus × strain interaction ($F_{8,136} = 2.4, P = 0.02$), but the main effects of strain, exposure diet, and sex were nonsignificant, as were all other interactions ($P > 0.05$ in all cases). Post hoc tests revealed that the responses to glucose, maltose, D-phenylalanine, and Polycose were significantly larger in B6 compared with 129 mice ($P < 0.05$ in all cases), but the strains did not differ in their responses to the other stimuli. Thus, B6 mice exhibited significantly larger relative...
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NST responses than did 129 mice to most of the compounds thought to taste sweet to mice, as well as to Polycose, but not to any of the other stimuli. These strain differences were independent of sex and unaffected by dietary experience.

Discussion

Sucrose exposure and ingestive responses

Over the 3 days of exposure to the sucrose diet, mice from both strains consumed 14–18 g/day of the 500 mM sucrose solution. Although the mice compensated for the caloric intake from the sucrose solution by reducing daily chow intake, they nevertheless increased overall caloric intake. The lack of strain difference in intake of the 500 mM sucrose solution across the exposure period was expected based on a report by Sclafani (2007). Prior reports of differential daily intake of sucrose solutions by B6 and 129 mice were limited to concentrations <500 mM.

Exposure to the sucrose diet modified behavioral responsiveness of the mice to suprathreshold concentrations of sucrose (500 mM) and saccharin (20 mM). However, the nature of the modifications varied as a function of mouse strain and ingestive response. That is, sucrose exposure enhanced the appetitive response to sweeteners in 129 mice and diminished it in B6 mice, but had no impact on the consummatory response in either strain. The observation that sucrose exposure selectively altered 1 component of the ingestive response has precedence. For instance, fetal ethanol exposure modified lick rates for chemical stimuli, but not the number of trials initiated in rats (Youngentob and Glendinning 2009; Glendinning et al. 2012). Other types of manipulations (e.g., overexpressing neuropeptide Y in the dorsomedial hypothalamus, overexpression of dopamine D2 receptors, systemic serotonin administration, and roux-en-Y gastric bypass surgery) modified number of trials initiated, but not lick rates in rats and mice (Mathes and Spector 2011; Mathes et al. 2012; Ward et al. 2012; Treesukosol et al. 2013). Our findings resemble the latter type of observation, except that sucrose exposure selectively altered trial initiation in the B6 and 129 strains in diametrically opposed ways.

Elsewhere, Sclafani (2006a) examined the impact of sucrose exposure on sucrose appetite in both 129P3 and B6 mice, using a progressive-ratio licking paradigm. He found that sucrose exposure significantly increased operant licking for approximately 500 mM (16%) sucrose in 129 mice, but decreased it in B6 mice. This finding is relevant to the present study for 3 reasons. First, we used the same sucrose-exposure regime and similar sucrose and saccharin concentrations in our lick tests. Second, even though Sclafani (2006a) used a different substrain of 129 mouse than us (i.e., 129P3), he established elsewhere (Sclafani 2007) that both substrains show a similarly robust sucrose exposure–induced increase in preference for (and intake of) sucrose. Third, our measure of appetitive responsiveness to the sweetener solutions (i.e., number of trials initiated) is analogous to progressive-ratio licking because it reflects the extent to which the mice actively and repeatedly sought out the sweetened solutions. Accordingly, we propose that our results complement those of Sclafani (2006a), and further strengthen the hypothesis that sucrose exposure increases sweetener appetite in 129 mice, but decreases it in B6 mice. Further studies are needed to explain this novel finding.

The results of the present study would seem to contradict the prior observation that sucrose exposure increases the preference for (and intake of) sweeteners in 23-h intake tests.
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However, this apparent contradiction merely underlines the fact that multiple mechanisms control sweetener intake and that changes in 1 facet of the ingestive response to sweeteners (e.g., its attractiveness) do not necessarily influence daily patterns of intake (Sclafani and Ackroff 2012). For example, the magnitude of the peripheral taste response reliably predicts the rate at which B6 mice lick for sweetener solutions, but not their daily intake of the same solutions (Glendinning et al. 2010). Likewise, overexpression of striatal D2 receptors increases the attractiveness of evaporated milk to B6 mice (as measured by a progressive-ratio task), but not daily intake of the same solution (Ward et al. 2012). Indeed, the available evidence indicates that long-term patterns of sweetener intake are determined to a large extent by postoral nutritional stimulation (Sclafani and Ackroff 2012), which would have been minimized in our 30-min test sessions.

Effect of sucrose exposure on taste-evoked multiunit responses of the NST

We found that B6 mice generated larger NST responses to sweeteners than did 129 mice and that the strain differences were unaffected by sucrose exposure. Analogous strain differences were reported previously in a study that measured single-unit NST responses of sugar-naive mice (C57BL/6ByJ and 129P3/J) (McCaughey 2007). Compared with 129P3 mice, the single-unit NST responses of B6 mice were significantly larger for sucrose, maltose, and d-phenylalanine, but not for NaCl, HCl, quinine, or IMP. The only discrepancies between the present results and those from the earlier study involved saccharin. Here, we report that B6 mice exhibited significantly larger multiunit NST responses to saccharin across a range of concentrations (i.e., 10–50 mM) relative to 129 mice, but no such strain difference was observed in the single-unit NST responses to 10 mM saccharin. We did not observe strain differences in responses to glycine in both the present and the earlier electrophysiological study. Although the taste of glycine is thought to have a sweet component in mice, there are also nonsweet components; further, glycine taste transduction is thought to function independently of the T1r2/T1r3 receptor (Eylam and Spector 2004; Inoue et al. 2007). We observed a significant difference in response magnitude between B6 and 129 mice for Polycose at 30%; in prior work involving multiunit CT recordings, C57BL/6ByJ mice exhibited significantly larger responses than 129P3/J mice to 10% (but not 30%) Polycose (Inoue et al. 2001).

Individual neurons in the NST can be classified according to their response characteristics (e.g., as sucrose-responsive S-cells or salt-responsive N-cells) and one can analyze the temporal or across-neuron patterns of firing of these neurons. For the current study, we ignored these attributes of the NST response because they did not seem to contribute to the previously observed strain differences between sucrose-naive B6 and 129 mice (McCaughey 2007). For example, although

Figure 2 Typical NST responses of a 129 mouse after 3 days of exposure to the control (top row) or sucrose (second row) diet and a B6 mouse after exposure to the control (third row) or sucrose (fourth row) diet. Responses to the sucrose concentration series are shown, along with the preceding and following responses to the 100 mM NH4Cl reference stimulus. For each response, the raw voltage is shown at the bottom, and above it is the integrated voltage that was used to quantify response sizes. All traces were drawn with the same horizontal scale (see lower left for scale bar), and vertical scales were kept constant for all responses for a given mouse.
S-cells were more common in B6 than 129 mice, the S-cells in both strains exhibited similar mean firing rates and temporal patterns of response to sucrose. The major difference between the single-unit NST responses of naive B6 and 129 mice was in the overall magnitude of their neural responses to sweeteners. For this reason, we hypothesized that the most parsimonious way for sucrose exposure to increase the acceptability of sweeteners to 129 mice (relative to B6 mice) would be to increase the overall level of NST response to them; this would presumably increase the intensity of their sweet taste. Accordingly, we selected a multiunit technique that has been evaluated specifically on its relevance to taste intensity perceptions in rats (Giza and Scott 1987). Although there are some caveats about this approach (e.g., it measures responses of some but not all taste-responsive neurons in the NST and does not provide information about response properties of different subclasses of NST cell), it appeared to do a good job of sampling broadly across NST cells. For instance, each recording site generated clear responses to chemicals that represented all taste qualities. Furthermore, our between-subject variability was not large enough to obscure clear differences between the B6 and 129 strains in the size of their sweetener responses.

The NST is a critical site of early taste processing, which integrates input from all parts of the oral cavity. Although it is more common to record from individual taste nerves, these recordings are limited by the fact that they reflect only a fraction of the overall gustatory signal that is sent to the brain. This is especially relevant given evidence that each oral region uses a different combination of proteins to transduce sweet taste in mice (Tizzano et al. 2008; Tomonari et al. 2012). Furthermore, no single peripheral taste nerve can be considered the sole mediator of the perception of sweetness. The CT nerve is known to be important in taste-guided behavioral responses to sweeteners, based on a positive relationship between CT nerve responses sizes and brief-access licking responses in mice (Glendinning et al. 2010). However, the greater superficial petrosal nerve evokes larger responses to sweeteners than does the CT in mice (Tomonari et al. 2012).

If sucrose exposure modified the initial transduction events for sweeteners, then we should have observed changes in the

**Figure 3** Relative NST responses of 129 and B6 mice to (A) a series of sucrose and saccharin concentrations and (B) 500 mM glucose (Glu), 500 mM maltose (Mal), 30% Polycose (Poly), 100 mM d-phenylalanine (Phe), 100 mM glycine (Gly), 100 mM NaCl (NaCl), 10 mM HCl (HCl), 10 mM IMP, and 20 mM quinine HCl (Qui). We show responses of B6 and 129 mice exposed to the sucrose (Suc) or control (Con) diet. ANOVAs indicated significant effects of strain and/or interactions between strain × chemical type, but there were no significant main effects of exposure diet (or interactions of exposure diet × strain). Post hoc tests were therefore conducted to compare mouse strains on responses to individual stimuli, with exposure conditions combined for a given strain. We indicate significant differences by placing different letters (a, b) above the bars corresponding to each strain (P < 0.05). N = 6–7 mice per strain and exposure diet.
overall strength of the neural signal in the NST. The fact that no such changes were observed suggests that the early stages of taste processing were unaffected by sucrose exposure. This finding agrees with a prior study reporting that dietary exposure to sucrose fails to alter CT nerve responsiveness to sucrose in rats, although this same study did report a reduction in expression of mRNA for T1r3 (Coleman et al. 2011). The larger taste-evoked NST responses of B6 mice to sweeteners likely reflect the fact that T1r3 in B6 mice binds with higher affinity to sweeteners than does T1r3 in 129 mice (Nie et al. 2005). Technically, we cannot rule out a strain difference in other central gustatory nuclei as contributing to our results, given that the NST receives descending influences from areas such as gustatory cortex in rodents. However, such descending fibers are thought to exert mainly a modulatory role on NST response sizes, and the pronounced size of our strain differences appears inconsistent with the sole action of such a mechanism.

Conclusions

Our results provide further support for the idea that dietary experience can substantially alter the contribution of genetics to sugar intake. For example, in the present study, B6 mice initiated more trials than did 129 mice for the sucrose and saccharin solutions before sucrose exposure. However, after sucrose exposure, 129 mice exhibited an equivalent (or even greater) number of trials than did B6 mice for the same sweetener solutions. Likewise, even though sugar-naive B6 mice were more attracted than sugar-naive 129 mice to specific sucrose concentrations in both 24-h intake (Sclafani 2006b) and fixed-ratio operant (Sclafani 2006a) tests, these strain differences were attenuated markedly by dietary sucrose exposure.

One of the primary goals of this study was to link diet-induced changes in appetitive or consummatory components of the ingestive response to changes in NST responsiveness. Accordingly, we treated the animals nearly identically in the 2 experiments. The only exception was that the mice were water- and food-restricted in Experiment 1 but not in Experiment 2. However, there is no reason to believe that this methodological difference rendered the 2 experiments incomparable because it was reported previously that food- and water-restriction schedule does not enhance or diminish taste-mediated licking responses of mice to sweeteners (Glendinning et al. 2005). Further, prior studies have established that sucrose exposure alters ingestive responsiveness to sweeteners in B6 and 129 mice that had free access to food and water (Sclafani 2006a, 2006b).

Sucrose exposure did not alter NST responses to sweeteners, despite inducing changes in appetitive responsiveness to sucrose and saccharin in Experiment 1 and altering other measures of sucrose intake elsewhere (Sclafani 2006a, 2006b, 2007). Presumably, then, sweeteners continued to evoke a more intense taste in B6 than 129 mice, regardless of experience. This suggests that the effect of sucrose exposure on the appetitive response was not mediated in the periphery or the NST. Rather, sucrose exposure must have altered responses to the sweeteners in higher areas of the gustatory neuraxis, to which the NST sends projections directly or indirectly: for example, the thalamus and cortex, which function primarily in stimulus identification, or the ventral forebrain, which functions primarily in ingestive motivation (Spector and Glendinning 2009). Given prior studies linking sucrose appetite to dopaminergic activation of the ventral forebrain structures (Hajnal et al. 2007, 2008; Sclafani et al. 2011; Salamone and Correa 2012), the most parsimonious explanation for our results is that sucrose exposure modulated dopaminergic activity of these structures in diametrically opposed ways in the B6 and 129 mice, leading to the observed changes in appetitive responsiveness.

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