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A Brief-access Test for Bitter Taste in Mice

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Abstract

Inbred mouse strains vary in their response to bitter-tasting compounds as assessed by 48 h preference tests. These differences are generally assumed to result from altered gustatory function, although such long-term tests could easily reflect additional factors. We developed a brief-access taste test and tested the responses of two inbred strains, as well as C3.SW congenic mice, to the bitter stimulus sucrose octaacetate (SOA). Water-deprived trained mice were tested with five concentrations of SOA (0.00018–0.18 mM) and distilled water in a Davis MS-160 apparatus. Trials were 5 s in duration and stimuli were presented randomly within blocks; each stimulus trial was preceded by a water rinse trial. Each concentration was presented twice in a session and mice were repeatedly tested across consecutive days. SOA-taster mice, including the SWR/J (SW) inbred and C3.SW congenic taster (T) mice, avoided licking SOA at concentrations >0.003 mM. In comparison, C3HeB/FeJ (C3) and C3.SW demitaster mice (D) licked all concentrations at the same rate as water. Concentration–response functions were similar across strains for both the brief-access test and a parallel 48 h preference test run on separate groups of mice. Furthermore, concentration–response functions were similar whether or not the brief-access test was preceded by a 4 day, single concentration pretest with SOA. The brief-access test is a suitable assay for bitter taste function in mice because it minimizes possible post-ingestive influences on taste.

Introduction

Genetic variation exists among various strains of laboratory mice for solution preference or aversion, as measured by fluid intake tests (Fuller, 1974; Hoshishima *et al.*, 1961; Lush, 1991; Whitney and Harder, 1994). Such natural variation has fostered physiological, biochemical and molecular approaches aimed at elucidating taste transduction mechanisms and the identity of genes underlying these mechanisms (Spielman *et al.*, 1996; Bachmanov *et al.*, 1997; Frank and Blizard, 1999; Miyamoto *et al.*, 1999; Chandrashekar *et al.*, 2000; Inoue *et al.*, 2001). Furthermore, taste transduction components and putative sweet and bitter taste receptors have been cloned (McLaughlin *et al.*, 1992; Adler *et al.*, 2000; Matsunami *et al.*, 2000; Kitagawa *et al.*, 2001; Max *et al.*, 2001; Montmayeur *et al.*, 2001; Sainz *et al.*, 2001), and mice with taste-related targeted gene deletions and insertions have begun to appear (Wong *et al.*, 1996). These advancements make necessary the development of taste salient behavioral assays for examining taste phenotypes among mice.

Genetic studies have generally depended on intake in single bottle or two-bottle tests as the dependent measure. These procedures are particularly amenable for testing large numbers of mice required for quantitative analysis. However, it is questionable whether these tests provide valid indicators of an animal's ability to recognize or discriminate a substance based on gustatory cues. This limitation has been recognized in studies with rats, and differences between intake tests and brief-access tests have been demonstrated. For example, the concentration–response function from a 24 h intake test for sucrose in rats has an inverted U shape, with intake peaking at intermediate concentrations. In a brief-access experiment (30 s trials), however, rats will increase licking with increased sucrose concentration in a monotonic fashion (Smith, 1988). Further, the lick rate for sucrose corresponds closely to the physiological response in the greater superficial petrosal nerve (Nejad, 1986), which is the peripheral taste nerve most responsive to sweet-tasting stimuli. This correspondence supports the notion that lick rate in a brief-access test reflects gustatory processing, independent of other controls of appetitive behavior. To date, only a few studies have been published that examine licking behavior in mice (Horowitz *et al.*, 1977; Harder *et al.*, 1984; Ninomiya and Funakoshi, 1989).

The salient feature of a brief-access taste test is the presentation of a taste stimulus for brief-duration trials (typically 5–30 s), and the dependent measure is the number

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of licks an animal makes in a trial (Grill *et al.*, 1987). In brief-access tests with aversive stimuli, water deprivation is commonly used to motivate licking behavior, and to provide a baseline of licking from which a concentration-dependent decrease can be measured. Depending on the testing apparatus, multiple concentrations of a taste stimulus may be presented, and a concentration–response function for an individual mouse can be constructed with the data from just one or a few test sessions. In comparison, concentration series in 24- or 48 h intake tests may take a week or more. Because the trials are brief, and because immediate responses are measured, intake of taste stimuli during a test session is limited and post-ingestive factors such as those associated with satiety or toxicity are greatly reduced or avoided. Thus, brief-access tests may be especially useful for measuring taste behavior to bitter-tasting compounds, for which toxicity often increases with concentration.

Based in part on methods from previous short-term taste tests with rats, we developed a brief-access procedure for mice using a commercially available taste-testing apparatus, the Davis MS-160 (Smith, 2001). We sought to compare results from this procedure with results from a two-bottle intake assay for sucrose octaacetate (SOA), a relatively common and non-toxic bitter taste stimulus. SOA is avoided in two-bottle tests by some strains of mice; this sensitivity is determined by allelic variation at a single genetic locus (Warren and Lewis, 1970; Lush, 1981). This locus, *Soa*, was mapped to a position on mouse chromosome 6 that was later determined to be the region where the T2R family of receptors is located (Capeless *et al.*, 1992; Adler *et al*., 2000; Matsunami *et al*., 2000). Despite this concordance, the exact sequence and gene product of *Soa* has yet to be determined. A congenic strain, C3.SW-*Soa*^{a}, was developed from SWR/J (SW: SOA taster) and C3HeB/FeJ (C3: SOA demitaster) inbred strains (Boughter and Whitney, 1995, 1998). In two-bottle tests, SW mice avoid concentrations of SOA as low as 0.001 mM. In contrast, C3 mice respond indifferently to all concentrations except a near-saturated 1 mM, for which they show a relative avoidance (demitasters are still significantly less sensitive to 1 mM SOA than tasters; the third phenotype, non-taster, is indifferent at this concentration). The C3.SW-*Soa*^{a} congenic taster mouse strain contains the SOA taster allele transposed on a ~99% C3 genomic background, and the SOA taster allele causes SW-like behavioral aversion to SOA. Significantly, these congenic mice have been maintained as heterozygotes for *Soa* by repeating generations of backcrossing phenotypic C3.SW tasters with C3 inbred mice. This means that each new generation of C3.SW backcross mice must be behaviorally tested with SOA in order to determine phenotype; each backcross mouse stands a 50:50 chance of being a phenotypic taster (T) or demitaster (D).

We took advantage of this previously studied SOA congenic system to test our brief-access procedure. We were interested in the ability of this procedure to produce in mice reliable concentration–response functions in response to the aversive taste of SOA. Furthermore, we compared the brief-access functions directly with those produced by two-bottle tests. Finally, because the C3.SW backcross mice must be tested to determine phenotype, we examined the ability of our assay to be used as a classifying procedure.

Methods

Animals

A total of 93 adult inbred (C3 and SW) and C3.SW N_{29}–N_{32} congenic taster (T) and demitaster (D) mice (*Mus domesticus*) were used in all experiments. All mice were naïve at the start of each experiment. The total numbers of mice per strain, per experiment are listed in Table 1 along with information about gender, age and weight. Overall, equal numbers of males (n = 46) and females (n = 47) were tested, and the age of the mice at the start of each experiment ranged from 49 to 158 days, with a median age of 72 days. Care was taken to age-match the strains within each experiment as well as possible. Previous studies have not indicated effects of gender on SOA or quinine aversion in mice (Lush, 1984; Whitney *et al.*, 1991; Harder *et al.*, 1992). Inbred mice were either laboratory bred or obtained from the Jackson Laboratory (Bar Harbor, ME). All T or D mice were laboratory bred (see below). Food (Harlan teklab, 7012) and water were available *ad libitum*, except where noted below.

C3.SW Congenic mice

The development of C3.SW-*Soa*^{a} heterozygous congenic taster mice has been described in detail previously (Boughter and Whitney, 1995, 1998). Through a protracted process of phenotypic selection (two-bottle tests with SOA) combined with lineal backcrossing across 11 generations, the dominant *Soa*^{a} (taster) allele was transferred from the SW donor strain on to the genomic background of the C3 inbred partner strain. In each backcross generation, ~50% of C3.SW mice were T and 50% were D mice, consistent with expectations from a one-locus model with an autosomal allele conferring a dominant taster phenotype. By generation N_{12}, each T mouse carried one copy of the *Soa*

Table 1 Total number of mice used in all experiments, along with gender, body weight and age

Strain	No. of mice		Body weight		Median age (days)
	Female	Male	Female	Male	
SW	8	13	17.14	24.37	78
C3	12	8	20.35	24.73	63
C3.SW T	15	10	24.39	28.53	72
C3.SW D	10	17	23.79	30.18	75
Total	47	46	21.89	27.46	72

taster allele and was in theory 98.9% genetically identical to the C3 inbred strain for genetic material not linked to the *Soa* locus (Flaherty, 1981). Further generations of C3.SW mice were developed, including generations N_{29}–N_{32} for use in the present study. We used 52 C3.SW mice, and as previously (Boughter and Whitney, 1998), the phenotype of these mice (T or D) was determined or confirmed within each experiment via two-bottle, 48 h tests with SOA (see Results). Twenty-five mice were classified as T and 27 as D mice; this 50:50 ratio was consistent with previous generations.

Two-bottle tests

Two-bottle preference tests were conducted in plastic cages (28 × 17.5 × 13 cm) with corn-cob bedding and stainless steel wire lids. Mice were housed individually, and food was available *ad libitum*. Two inverted graduated cylinders were placed on either side of a cage lid, and each cylinder had a neoprene stopper with a straight stainless-steel sipper tube that protruded into the cage about 6 cm from the cage floor. One cylinder contained 0.1 mM SOA, whereas the other contained distilled water. After 24 h the amount consumed from each cylinder was recorded and the position of the cylinders was switched. Amounts consumed were again recorded after 24 h. A preference ratio (PR) (amount of solution consumed/total amount consumed) was calculated for each day and the two were averaged to obtain a 48 h PR for each mouse.

Brief-access tests

Brief-access tests were conducted in a Davis MS-160, a commercially available taste test apparatus (DiLog Instruments, Inc., Tallahassee, FL). The mouse was placed in a rectangular test cage (30 × 14.5 × 16 cm) with a stainless steel mesh floor, and could access taste solutions or water via a small opening in the front wall of the chamber. Access to stimuli was computer controlled. A given trial began when a shutter was opened to allow access to a stainless steel sipper tube (2.5 cm from floor), and ended after a defined time period when the shutter closed. In between trials, the computer drove a stepping motor to position one of up to 16 drinking tubes in front of the stimulus-access opening in preparation for ensuing trials. Licks were counted with a high-frequency AC contact circuit.

For each brief-access experiment, water-deprived mice were first trained to lick water in the Davis apparatus, then tested in the apparatus with a concentration series of SOA plus water. Approximately 24 h prior to training, water bottles were removed from the home cages of individually housed mice. On the first training day, a mouse was placed in the test chamber and given access to distilled water for 30 min. The amount consumed was recorded and mice were returned to the home cage. On the second day, the procedure was repeated, and the number of licks in the 30-min session was recorded.

Testing occurred on days 3–5. During test sessions, the mouse initiated a 5 s trial with a single lick on the sipper tube. The time between the opening of the shutter and the first lick was recorded as the 'latency to lick'. In the event that the mouse did not make a lick within 300 s of the shutter opening, the shutter was closed and the next trial begun. There were two types of trials: water rinse trials and test trials. Each test trial was preceded by a water rinse trial. The rationale for using water rinse trials was to minimize carryover effects from one trial to the next (e.g. after-taste or contrast effects). Test trials consisted of the presentation of either water (water test trial) or one of five concentrations of SOA (¾ log steps: 0.00018, 0.001, 0.006, 0.03 and 0.18 mM), which were prepared daily from a reagent grade source (Sigma, St Louis, MO) and distilled water. The presentation order of test stimuli was randomized in two blocks of six; with rinse trials, the mouse could initiate up to 24 trials a day. A 15 s intertrial interval separated these 24 trials.

Data analysis

Number of licks for each SOA trial, plus water test trials, were averaged across the three test sessions for each individual mouse. These data were then reported as lick ratios (average number of licks to SOA/average number of licks during water test trial). This served to control for potential differences in lick rate that were non-gustatory in origin; i.e. the speed of the central pattern generator (CPG) for licking or the overall activity of the strain or individual. It is important to note that the water rinse trials were not considered in the calculation of lick ratios for SOA, although these trials were considered later in the analysis of water licking (e.g. Table 2). For concentration series, mean lick ratio and PR data were fitted with sigmoidal three-parameter functions: $f(x) = (1 - d)/(1 + (x/c)^b) + d$, where x represents SOA concentration, b represents the slope, c represents the concentration of SOA that evoked the half-maximum response, and d represents the asymptotic minimum. Parameter c was compared between strains with t-tests. Additionally, mean data were compared between strains using one- or two-way analyses of variance, with a repeated measures design and post-hoc comparison tests (Scheffé) where appropriate. The statistical rejection criterion (α) for all tests was set at the 0.05 level.

Experiment 1: A–B–A design

In this experiment, nine C3, nine SW and 13 C3.SW N29 congenic mice were given two consecutive 48 h two-bottle tests with 0.1 mM SOA during the first week. The purpose of these 'pretests' was to classify the C3.SW mice as T or D prior to brief-access testing. At the conclusion of these tests, mice were placed in home cages with *ad libitum* access to water and chow for 2 days. Water bottles were then removed from the home cages, and the mice were trained (2 days) and tested (3 days) using the brief-access procedure. During this week, mice received their daily fluid intake during the

Table 2 Licks during water rinse and water test trials

Strain	Mean licks	Maximum licks[a]
(A) A–B–A Design		
SW	42.5 ± 1.51	53.4 ± 0.96
C3	36.8 ± 1.21[b]	48.0 ± 0.44[b]
T	37.7 ± 0.78	46.9 ± 0.91[b]
D	32.4 ± 1.28[b]	47.2 ± 1.11[b]
(B) B–A design		
SW	52.4 ± 0.89	59.8 ± 0.63
C3	33.4 ± 1.88[b]	51.3 ± 0.67[b]
T	37.4 ± 3.16[b]	50.0 ± 1.0[b]
D	32.5 ± 1.32[b]	52.0 ± 0.75[b]

[a]The average (across subjects) of the highest number of licks produced on any single water rinse or water test trial.

[b]Differs significantly from SW strain (Scheffé, $P < 0.05$).

training and testing sessions. After the last test session, water bottles were replaced on the home cages, and after two more days they were again given two consecutive 48 h two-bottle tests with 0.1 mM SOA.

Experiment 2: B–A design

In this experiment, three C3, four SW and 12 C3.SW N31 or N32 mice were given the brief-access tests in the first week, and two-bottle tests in the second week. There were two reasons for this design: first, we examined whether C3.SW T and D mice could be reliably classified on the basis of the brief-access test alone. The SOA phenotypes of these mice, as well as the inbred strains, were then verified based on the results of the two-bottle post-test. Second, this protocol allowed us to compare lick ratios in naïve mice versus those with prior experience with SOA (A–B–A design).

Experiment 3: two-bottle concentration series

Five C3, five SW and 17 C3.SW N30 mice were given consecutive 48 h two-bottle tests with the same concentration range (0.00018–0.18 mM, ¾ log steps) of SOA that was used in the brief-access tests. This experiment was repeated with a second group of mice from all three strains (three C3, three SW, 10 C3.SW N32) and for analysis data were collapsed across both experiments. C3.SW mice were classified as T or D on the basis of their PRs to 0.13 mM SOA; this classification was verified with a single 48 h post-test with 0.1 mM SOA. This experiment was conducted in order to make a direct, concentration-by-concentration comparison of the brief-access and two-bottle procedures.

Results

A–B–A design

Pre- and post-tests

Individual PRs and strain means for the two-bottle pretest

Table 3 PRs for 0.1 mM SOA pre- and post brief-access testing (A–B–A) design

Strain	Pre-test		Post-test	
	1	2	1	2
SWR/J	0.01	0.02	0.07	0.02
	0.02	0.02	0.04	0.04
	0.01	0.04	0.05	0.04
	0.04	0.02	0.02	0.04
	0.04	0.04	0.06	0.04
	0.04	0.02	0.05	0.04
	0.03	0.02	0.04	0.03
	0.00	0.03	0.04	0.05
	0.02	0.03	0.05	0.04
Mean	0.02	0.03	0.05	0.04
C3HeB/FeJ	0.67	0.64	0.50	0.41
	0.72	0.66	0.39	0.52
	0.44	0.57	0.44	0.50
	0.54	0.62	0.49	0.41
	0.31	0.48	0.59	0.37
	0.63	0.40	0.58	0.42
	0.53	0.48	0.55	0.53
	0.44	0.51	0.41	0.45
	0.50	0.53	0.63	0.51
Mean	0.53	0.54	0.51	0.46
C3.SW T	0.10	0.05	0.05	0.02
	0.06	0.02	0.08	0.05
	0.04	0.05	0.06	0.04
	0.07	0.10	0.09	0.07
	0.06	0.04	0.06	0.05
	0.07	0.02	0.03	0.05
	0.08	0.06	0.05	0.06
Mean	0.07	0.05	0.06	0.05
C3.SW D	0.28	0.61	0.31	0.46
	0.32	0.42	0.42	0.44
	0.50	0.51	0.47	0.48
	0.49	0.48	0.45	0.46
	0.53	0.29	0.53	0.54
	0.50	0.60	0.56	0.62
Mean	0.44	0.49	0.46	0.50

are shown in Table 3. For both test periods all SW mice dramatically avoided SOA (PR < 0.05), whereas C3 mice were indifferent (PR ranged from 0.31 to 0.72). Based on previous studies with C3.SW congenic mice, we set a criterion PR to determine *Soa* phenotype among C3.SW mice: mice were classified as T if they had a PR of <0.15, D if ≥0.15 (Boughter and Whitney, 1995). Using this criterion, 13 C3.SW mice were unambiguously classified as T (seven) or D (six) mice (Table 3). Across both test periods, SW and T mice avoided 0.1 mM SOA in a similar fashion, whereas C3 and D mice were indifferent; the strain difference was significant [two-way ANOVA, $F(3,27) = 175.27$; $P < 0.001$]. During preference testing, SW mice consumed more total

fluid (mean = 13.1 ml) than the other three strains (C3 = 8.9, T = 10.2, D = 9.0) during each 48 h test. This strain difference for total fluid intake was significant [two-way ANOVA, $F(3,27) = 14.65$; $P < 0.001$]. T mice differed from D mice in terms of SOA aversion, but not in total fluid intake, indicating that the *Soa* taster allele did not influence the latter measure.

Table 3 also contains results from the post-test. In every case, PRs for individual mice were virtually identical to those in the pretest, with all mice easily meeting the criterion value. In addition, total fluid intake was similar to the pretest (data not shown).

Brief-access test

In the brief-access test, SW and T mice avoided SOA in a concentration-dependent manner, whereas C3 and D mice were indifferent to SOA (Figure 1). A two-way ANOVA (strain × concentration) indicated significant effects for strain [$F(3,27) = 50.97$; $P < 0.001$], concentration [$F(4,108) = 72.10.2$; $P < 0.001$], and the strain × concentration interaction [$F(12,108) = 25.56$; $P < 0.001$]. Post-hoc tests (Scheffé) confirmed SW and T mice did not differ from one another but did differ from C3 and D mice. The latter strains did not differ. The concentration of SOA that evoked the half-maximum avoidance response, as calculated from the functions fitted to the SW and T data, did not differ significantly.

Latency to first lick. We also examined the latency to first lick in each trial, because concentration-dependent changes in this measure have been shown previously in the Davis apparatus to be indicative of olfactory contributions to sucrose responsiveness (Rhinehart-Doty *et al.*, 1994), and because another study suggested that animals may be able to smell the bitter compound quinine (Benjamin, 1960). Latency did vary significantly with SOA concentration [Figure 2A; $F(5,135) = 2.47$; $P < 0.04$], although there was not a significant effect of strain. When latencies for each concentration were collapsed across strain, it was evident that mice had a somewhat shorter latency to lick during the water test trials, and during trials with 0.00018 mM, than during trials with 0.001–0.18 mM SOA (Figure 2B). This possibly indicated that mice could smell or otherwise anticipate the higher concentrations of SOA, or conversely could anticipate the water and the most diluted SOA concentration. In either case, the latencies for the higher four concentrations were roughly equal, indicating that the mice could not distinguish SOA concentration with a non-taste cue. Mice of all strains took about 10–20 s to make initial contact with the spout. However, this varied considerably within each set of trials; mice tended to have a shorter latency earlier in the test session when they were thirstier. Collapsed across both strain and concentration, the mean latency for trials 1–12 was 8.27 s, whereas the mean latency in trials 13–24 was 22.20 s.

Differences in water lick rate. Although SW and T mice displayed similar responses to SOA (Figure 1), when water test trials and water rinse trials were examined, we found that SW mice actually licked water at a higher rate than the other strains [Table 2A; $F(3,27) = 9.9$, $P < 0.001$]. This

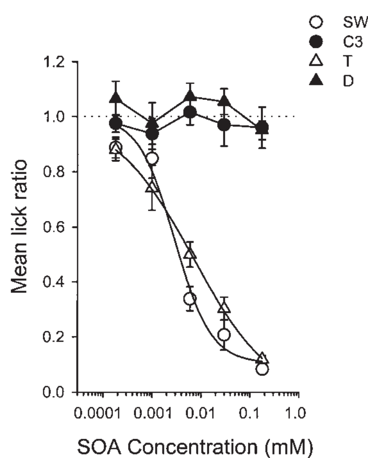

Figure 1 Mean lick ratios for each strain, A–B–A design. For SW and T mice, lick ratios decreased with increasing concentration. Mean data from these strains were fit with logistic 3-parameter functions. These functions reflect the fact that mice from both of these strains make fewer licks to SOA at higher concentrations during a 5 s trial. C3 and D mice licked all SOA concentrations at roughly the same rate as water.

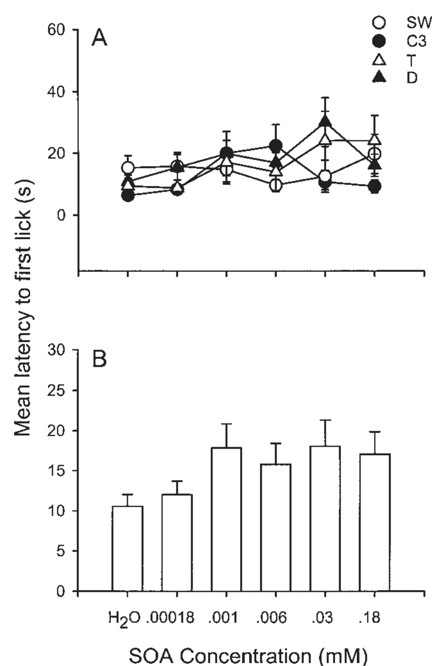

Figure 2 **(A)** Mean latency to first lick during brief-access SOA testing. Mice from each strain displayed similar latencies to either water or SOA during testing; there was a significant effect of concentration, but not strain. **(B)** Mean latency collapsed across strains. Mice had a shorter latency to water and 0.00018 mM SOA than to the higher concentrations of SOA.

suggested that SW mice might be capable of producing more licks in the 5 s taste trial than mice from other strains, which was further supported by comparing the maximum number of licks produced by mice from each strain (Table 2A).

Because of the difference in licks to water, we hypothesized that SW mice may have a faster CPG for licking as compared with the other strains. To test this hypothesis, we examined the inter-lick interval (ILI) distribution for each strain during the 30 min training period with water (i.e. day 2). If the SW mice had a faster CPG, the interval between licks should generally be shorter than those of the other strains of mice (Horowitz *et al.*, 1977). The mean ILIs (per strain) for each 5 ms bin, expressed as a percentage of the total number of ILIs across all bins, is shown in Figure 3. Perhaps surprisingly, the strains did not differ in their ILI distributions: On average, each strain possessed a bimodal distribution of ILIs, with the mode occurring at about 110–115 ms. The second, much smaller, peak in the distributions, occurring at 190–220 ms, reflects incidents where mice 'missed' a lick in a given sequence, thus resulting in an ILI about double that of the normal.

A second possibility is that SW mice may be thirstier than mice from other strains. Body weight did not differ significantly as a function of strain (data not shown), although SW mice produced about twice as many licks as mice from other strains during the 30 min training session on day 2. Whatever the source for differences in lick rate to water, these differences in phenotype are not influenced by *Soa*, as T and D mice are far more similar to the C3 parental strain than the SW. These differences, while minor, underscore the appropriateness of expressing taste-responsivity as a ratio relative to each animal's average licking rate to water.

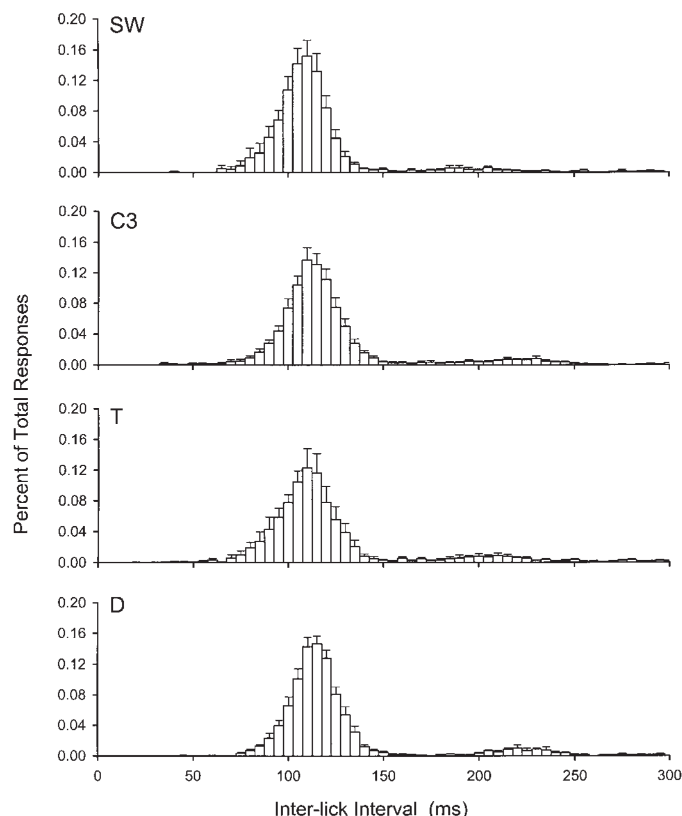

Figure 3 Mean interlick interval (ILI) distributions for each group of mice in the A–B–A experiment during the 30 min water training period. The distributions did not differ among strains.

B–A design

As in the A–B–A design, SW mice decreased their licking as a function of concentration, whereas C3 mice licked all concentrations at the same rate as water (Figure 4). Of 12 C3.SW mice, three individuals had decreased lick ratios at the higher concentrations and were subsequently classified as tasters after two-bottle post-tests with 0.1 mM SOA (Table 4). The remaining nine C3.SW mice possessed a mean lick rate that was similar to water for all concentrations of SOA. These mice were subsequently confirmed as demitasters by post-test results (Table 4). In either case, lick ratio functions for individual C3.SW mice (not shown) were clearly indicative of phenotype. A two-way ANOVA indicated significant effects for strain [$F(3,15) = 31.98$; $P < 0.001$], concentration [$F(4,60) = 30.08$; $P < 0.001$], and the strain × concentration interaction [$F(12,60) = 10.13$; $P < 0.001$]. Post-hoc tests (Scheffé) confirmed that SW and T mice possessed lower lick ratios than C3 and D mice. Two-bottle post-test results for all strains were well within taster–demitaster criterion (Table 4; cf. Table 3). As in the A–B–A test, the half-max parameter did not differ between SW and T mice. Analysis of latency did not reveal significant effects, in contrast to the modest effects seen in the A–B–A design.

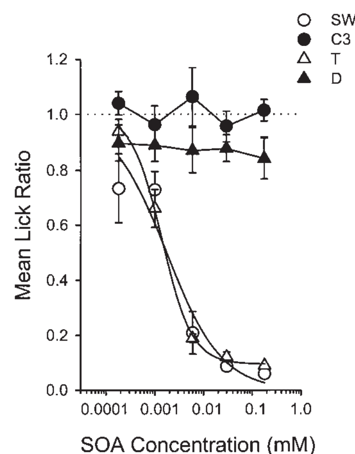

Figure 4 Mean lick ratios for each strain, B–A design. Lick ratios for SW and T mice decreased with increasing concentration. Mean data from these strains were fit with logistic three-parameter functions. In comparison, the concentration–response functions for C3 and D mice were flat, and indicative of non-tasting.

Table 4 Preference ratios for 0.1 mM SOA, post-test (B–A design)

Strain	1	2
SW	0.04	0.03
	0.03	0.03
	0.03	0.04
	0.04	0.04
Mean	0.04	0.04
C3	0.43	0.51
	0.57	0.64
	0.47	0.50
Mean	0.49	0.55
T	0.07	0.04
	0.04	0.05
	0.05	0.03
Mean	0.05	0.04
D	0.43	0.67
	0.36	0.54
	0.56	0.51
	0.58	0.42
	0.42	0.25
	0.40	0.59
	0.45	0.48
	0.57	0.57
	0.57	0.48
Mean	0.48	0.50

Water lick rate

As in the A–B–A experiment, SW mice licked at a higher rate than the other strains during the water test and water rinse trials [Table 2B; $F(3,15) = 26.8$, $P < 0.001$]. This strain difference was also manifest in maximum licks in a trial (Table 2B). Unlike the A–B–A experiment, however, there was a modest strain difference with regards to weight loss during water deprivation [$F(3,15) = 4.66$; $P < 0.02$]. Post-hoc tests indicated that SW mice differed only from D mice.

Preference testing with concentration series of SOA

Figure 5 contains the results of testing mice from each strain with an ascending series of SOA (0.00018–0.18). C3.SW mice were classified (after testing) as T ($n = 14$) or D ($n = 12$) on the basis of their PR at the highest concentration (0.18 mM), using the same criterion (PR <0.15 or ≥0.15) as the previous experiments. This classification was confirmed for all T and D mice with a single, 48 h post-test with 0.1 mM SOA (data not shown). Avoidance levels for the SOA concentration series were very similar to those obtained in brief-access testing (Figure 5; *cf.* Figures 1 and 3). Results from a two-way ANOVA showed significant effects of strain [$F(3,34) = 120.97$; $P < 0.001$] and concentration [$F(4,136) = 26.17$; $P < 0.001$]. There was also a statistically significant interaction [$F(12,136) = 19.12$; $P < 0.001$]. Post-hoc tests

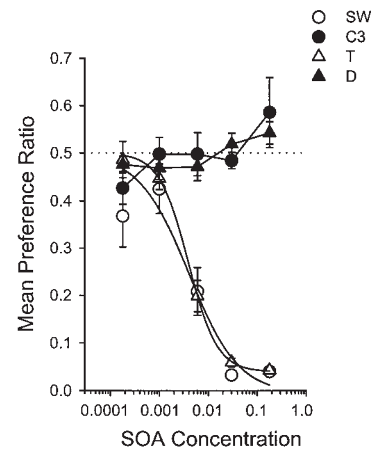

Figure 5 Mean PRs for each strain to the concentration series of SOA. PRs for SW and T mice decreased with increasing concentration. Mean data from these strains were fit with logistic three-parameter functions. In comparison, the concentration–response functions for C3 and D mice were flat, indicating indifference.

(Scheffé) confirmed that SW and T mice possessed lower PRs than C3 and D mice. As in the brief-access tests, the half-max parameter did not differ significantly between SW and T mice. In fact, this parameter did not differ significantly among either SW or T mice when the brief-access functions from the A–B–A and B–A tests were compared with the functions from the preference test, or with each other. This further illustrates the point that the level of aversion was similar for these two strains in either type of test.

Discussion

Lick ratio functions for SOA

In the first experiment (A–B–A), inbred and backcross mice were first ‘screened’ with consecutive 48 h SOA two-bottle preference tests, brief-access tested with a SOA concentration series, and then preference tested again. The initial screen confirmed that SW inbred mice avoided 0.1 mM SOA, whereas C3 inbred mice were indifferent. Additionally, the screen unambiguously revealed that seven of 13 C3.SW congenic backcross mice were phenotypic tasters (T); the remaining six mice were classified as demitasters (D). Concentration–response functions for each mouse, and mean functions for each strain, were then generated from 3 days of brief-access testing. SW and T mice avoided SOA in a concentration-dependent manner, whereas C3 and D mice licked all concentrations of SOA at the same rate as water. These results were consistent with expectations that variation at the *Soa* allele influences taste sensitivity to SOA itself, i.e. the T mice displayed a similar level of taste avoidance as the SW mice. Because the trials with SOA were only 5 s in duration, post-ingestive cues could not specifically affect an ongoing trial. Furthermore, because all stimuli (water and SOA concentrations) were presented intermixed

in a single test session, post-ingestive feedback could not differentially affect lick rate across stimuli. The results of the brief-access test can therefore be ascribed to gustatory processing with considerably more confidence than can the two-bottle tests. While this test offers these advantages over intake tests, it is important to acknowledge that other oro-sensory factors, such as somatosensation, could contribute to the behavioral differences observed. The hypothesis that the brief-access results are based solely on gustatory input could be examined with further studies combining behavior with nerve transection as has been done in the rat (St. John *et al*., 1994). After gustatory nerve transection, somato-sensory input would persist via the lingual nerve. If the concentration-based rejection in SW and T were reduced by the nerve cuts, a role for gustatory input in the observed behavior would be clarified.

The lick ratio functions for each strain were extremely similar to preference functions using the same concentration range (cf. Figures 1 and 5). Perhaps this is not surprising, given that SOA is a non-toxic aversive compound. We expect that the brief-access test will prove especially useful in testing those bitter-tasting stimuli that are toxic, such as the commonly used bitter taste stimuli caffeine and cyclo-heximide. There are few behavioral studies that have examined sensitivity to these compounds, either in rats or mice. However, one example is potentially instructive: Strains of mice differentially avoid phenylthiocarbamide (PTC) in two-bottle intake tests, but typically only after a few days of consumption (Whitney and Harder, 1986). It is thought that this aversion is due to a form of taste conditioning associated with the mild toxic effects of PTC intake. Preliminary data from our laboratory (unpublished data) suggest that mice did not avoid concentrations of PTC in a brief-access test that they will avoid in an intake test.

Classification of mice using brief-access test

In the A–B–A design, mice had experience with SOA during the preference test that conceivably might have altered SOA sensitivity in the brief-access test. We directly tested this possibility in a second experiment in which mice were first screened with the brief-access procedure, only then 'confirming' this classification with the traditionally used preference test. In fact, there was no evidence that the concentration–response functions differed substantially between the A–B–A and B–A conditions (Figures 1 and 4). The brief-access procedure allowed for discrimination of T from D mice. Because of recent advancements in molecular and genomic approaches to the taste system, there is great interest in taste-salient screening techniques for mice with taste-related targeted gene insertions and deletions. Taste behavior differences must be demonstrated in the absence of potential post-ingestive cues if the goal is to elucidate the molecular or physiological underpinnings of taste behavior. We believe that (at least for bitter stimuli) our brief-access procedure accomplishes this; the mere possibility that post-ingestive factors can influence the results begs for the use of the more taste-salient short-term test.

Multiple concentrations of a stimulus can be delivered in a single session using the Davis MS-160, and reliable concentration–response functions can be generated with a few days of testing. In contrast, it generally takes one or more weeks to collect a concentration–response function using a two-bottle assay. On the other hand, there is a practical limit to how many mice can be tested in a single day using the brief-access procedure, while two-bottle tests can be reasonably done with large groups of mice (i.e. 50–100), depending on the availability of testing equipment and space. Our procedure represents a more taste-salient approach, one that may be amenable for phenotypically screening smaller squads of mice (i.e. 10–20) in a single week. We suggest that the two-bottle testing paradigm be used as a screening tool only after it has been verified that, over a range of concentrations, the preference testing functions match those generated by a more taste-salient test, like the brief-access test shown here. This criterion is met for SOA in the present strains of mice.

Differences in water lick rate

Interestingly, there was a difference in the licks to water between strains: Inbred SW mice tended to lick water more than C3, T or D mice in either a 5 s trial or during a 30 min training session. This difference was not due simply to a faster lick rate, because the ILI distributions in a 30 min water trial were similar among strains (Figure 4). It was possibly due to a difference in thirst, although weight loss during water deprivation was similar between strains. Furthermore, SW mice differed from all other strains in a non-deprived state: SW mice had a greater total con-sumption of both SOA and water during consecutive 48 h two-bottle tests with 0.1 mM SOA. In any case, the *Soa* taster allele did not have an effect on water licking, or total consumption: T mice were similar to C3 and D mice, and dissimilar to SW mice. This finding also provides a rationale for presenting brief-access lick behavior in terms of lick ratios. The ratio corrects for any difference in a mouse's ability or tendency to make a certain number of licks in a given trial. After data are normalized in this fashion, SW and T mice have concentration–response functions that virtually overlap, reflecting the complete effect of the *Soa* taster allele on SOA taste sensitivity.

In summary, the brief-access test detailed here appears to be a useful tool for accurate assessment of taste function in mice. Because this test relies on water deprivation to motivate licking behavior, the test is appropriate for aversive stimuli, such as bitter-tasting stimuli or acids. Unlike two-bottle assays, the brief-access procedure allows a greater depth of analysis concerning taste behavior. In addition to the defining feature of lick ratio measurements in brief trials, this procedure offers the ability to examine micro-structural aspects of licking patterns during water training

(e.g. ILI distributions), examine latencies to discern possible olfactory contributions, to test a full range of concentrations in a single session, to test quickly trained mice on other stimuli of interest in the same or ensuing test sessions, and to make comparisons with intake tests in order to implicate post-ingestive influences.

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