Chem. Senses 27: 461–474, 2002

A High-throughput Screening Procedure for Identifying Mice with Aberrant Taste and Oromotor Function

John I. Glendinning, Jodi Gresack and Alan C. Spector[1]

Department of Biological Science, Barnard College, Columbia University, New York and [1]Department of Psychology, University of Florida, FL, USA

Correspondence to be sent to: John I. Glendinning, Department of Biological Science, Barnard College, Columbia University, 3009 Broadway, New York, NY 10027, USA. e-mail: jglendinning@barnard.edu

Abstract

Little is known about how specific genes influence taste function in mammals. One of the most promising ways to fill this void is to screen the progeny of chemically mutagenized (or genetically altered) mice for aberrant taste phenotypes and then identify the mutated gene(s) that is associated with each taste anomaly. To exploit this approach, a high-throughput and robust screening procedure is needed. We have attempted to meet this demand by developing an automated procedure that assesses taste responsiveness of individual mice to palatable and unpalatable taste stimuli. We focused on three taste stimuli (quinine hydrochloride, QHCl; sodium chloride, NaCl; and sucrose) and one mouse strain (C57BL/6). We used a commercially available gustometer system that both monitors the licking responses of mice and controls the presentation of each taste stimulus during successive 5 s trials. We describe a screening procedure that (after 2 days of simple training) can generate a concentration–response curve for NaCl or sucrose during a single 30 min test session, and for QHCl over three 30 min test sessions. A normative database based on the responses of 98 mice subjected to our screening procedure is also presented. We envision that investigators could use this normative database to assess taste function in the progeny of mutagenized (or genetically altered) mice. Any mouse that deviates significantly—e.g. three standard deviations (SD)—from the mean of the normative database would be flagged as having a potentially interesting mutation. We also developed an additional second screen for identifying mice with oromotor abnormalities. This latter screen is necessary because oromotor problems could lead to false positives or negatives in the screen for taste function, but is also useful for researchers interested in genes influencing oromotor circuitry. Throughout the development of the screening protocol, we sought to balance two conflicting demands: the need to maximize the screen's sensitivity and minimize its duration. This screen represents a significant improvement over the common two-bottle preference test because it assesses taste function more specifically and in a fraction of the time.

Introduction

Over the last decade, investigators have gained considerable insight into how taste stimuli are transduced, coded and processed (Glendinning *et al*., 2000; Smith and Davis, 2000). Comparatively little is known, however, about how specific genes influence the development and maintenance of these sensory processes, owing to complexities such as polygenic inheritance, locus heterogeneity and gene–environment interactions. One of the most powerful ways to circumvent these complexities is to screen the progeny of chemically mutagenized (or genetically altered) animals for aberrant phenotypes (Battey *et al.*, 1999; Nolan *et al.*, 2000; Wahlsten, 2001). This approach has already been used successfully in two species of invertebrate, *Caenorhabditis elegans* and *Drosophila melanogaster*, to identify single genes that influence anatomy, physiology and/or behavior (Dudai *et al.*, 1976; Bargmann, 1993; Sayeed and Benzer, 1996), but has been implemented only recently in mice (Nolan *et al.*, 1997; Sayah *et al.*, 2000; Wahlsten, 2001). For taste researchers to exploit this approach, it will be necessary to develop a high-throughput and robust procedure to identify mice with aberrant gustatory function.

The most commonly used taste phenotyping procedure, two-bottle preference testing, is both low-throughput, i.e. takes several days to assay a single concentration of a single taste stimulus, and nonspecific to taste, i.e. generates results that are influenced by postingestive and experiential factors (Grill *et al.*, 1987; Spector, 2000, 2002). In this paper, we describe a modification of the brief-access taste test for use as a phenotyping procedure in mice. The brief-access taste test involves the repeated presentation of various chemical stimuli for short periods of time (5–30 s) and has been used to measure taste responsiveness in rats (Young and Trafton, 1964; Davis, 1973; Krimm *et al.*, 1987; Smith *et al.*, 1992; Breslin *et al.*, 1993; Spector *et al.*, 1993, 1996; St John *et al.*,

1994) and mice (Boughter *et al.*, 2002) in a fraction of the time required for the two-bottle preference testing.

We believe that brief-access taste testing is an ideal procedure for detecting aberrant taste responses in mice because: (i) it measures immediate licking responses to very small stimulus volumes, reducing the contribution of non-gustatory factors; (ii) it focuses on motor output during the period when the stimulus first contacts the oral sensory receptors; (iii) it can generate a concentration–response function from a single mouse in a single test session; (iv) it provides a comprehensive and quantitative depiction of a mouse's behavioral responsiveness to different taste stimuli; (v) it generates more than one data point per concentration per animal, which improves the estimate of responsiveness; and (vi) it enables one to present taste stimuli in a randomized order during individual test sessions, which minimizes the occurrence of systematic contrast effects and the chances of mice forming associations between a given concentration of a taste stimulus and any possible postingestive effects.

As with all procedures, brief-access taste testing has some caveats. First, in some instances, one must place subjects on restricted schedules of water and/or food access to motivate them to sample taste stimuli. This should not be a problem, however, if these conditions are held constant across animals. In fact, such restricted food and water access schedules can be a strength because they produce subjects with a standardized physiological state that are highly motivated to learn and perform behavioral tasks (Desimone *et al.*, 1992; Hughes *et al.*, 1994; Heffner and Heffner, 1995). Second, one must use a relatively complex device (called a gustometer) both to monitor licking activity and to present taste stimuli according to a precise trial schedule. Relative to the cost of common biomedical testing equipment, however, gustometers are inexpensive (~US$5000 apiece). Third, brief-access taste tests rely on the hedonic characteristics of the taste stimulus to drive behavior. Although this is not the most ideal situation for the assessment of discriminative taste function (Spector, 2002), there is no way to overcome this issue without resorting to conditioning techniques, which are time-intensive and not well-suited for high-throughput screens.

In this study, we present a brief-access testing procedure for evaluating taste responses of individual mice to chemical stimuli that are considered palatable or unpalatable. We focused on three taste stimuli (quinine hydrochloride, QHCl; sodium chloride, NaCl; and sucrose) and one mouse strain (C57BL/6J). Although we considered using a wider array of taste stimuli, we felt that doing so would make the testing procedure screen prohibitively long. We chose QHCl, NaCl and sucrose because they stimulate different transduction mechanisms (Glendinning *et al.*, 2000), activate different populations of primary taste afferents (Smith and Frank, 1993) and appear to elicit qualitatively different taste perceptions (Nowlis *et al.*, 1980) and oromotor consummatory responses (Grill and Berridge, 1985) in rodents. In addition, previous work has identified a critical role of genetics in determining the responsiveness of mice to these compounds (Boughter *et al.*, 1992; Ninomiya and Funakoshi, 1993; Whitney and Harder, 1994; Wong *et al.*, 1996; Bachmanov *et al.*, 1997, 1998; Blizard *et al.*, 1999).

Our specific goals were: (i) to develop procedures for measuring taste responsiveness to unpalatable (QHCl and NaCl) and palatable (sucrose) taste stimuli; (ii) to determine the minimum number of test sessions that are required with each taste stimulus to generate reliable concentration–response functions in individual mice; and (iii) to generate a normative database of responses of wild-type C57BL/6J mice to the three taste stimuli, against which future studies could compare the responses of the progeny of chemically mutagenized mice with this genetic background.

Materials and methods

Subjects

We used C57BL/6J mice, obtained from the Jackson Laboratory (Bar Harbor, ME). All mice were housed individually in standard shoebox cages (27.5 × 17 × 12.5 cm) in a room with automatically controlled temperature, humidity and lighting (12 h:12 h light:dark cycle). Roughly equal numbers of males and females were used in each experiment, with individuals ranging in age from 7 to 9 weeks old. The sample sizes for each experiment are indicated in the figure legends. Except where noted otherwise, the mice were maintained on *ad libitum* Purina Laboratory Chow (5001) and water. The mice were naïve to the taste stimuli prior to the experiment and were tested during the light phase of their light–dark cycle.

Apparatus

We used a commercially available gustometer (Davis MS160-Mouse; DiLog Instruments, Tallahassee, FL) to record the licking behavior of each mouse. The gustometer consisted of a testing chamber (14.5 cm wide, 30 cm deep, 15 cm tall), a taste stimulus delivery system and a dedicated computer (with software that controlled the presentation of taste stimuli and recorded the precise timing of each lick). Once a mouse was placed in the test chamber, a motorized shutter opened (in <0.5 s). This provided the mouse with access to a sipper tube (connected to a fluid reservoir) through a slot (1.5 cm wide, 4.0 cm high) in the stainless-steel wall; the fluid reservoir (a 15–20 ml container) could contain any taste stimulus dissolved in deionized water. The gustometer automatically centered the appropriate sipper tube in the slot behind the shutter by moving the stimulus reservoir manifold laterally in precise steps. The stimulus delivery system could accommodate up to 16 different fluid reservoirs (i.e. taste stimuli) and the presentation schedule of each taste stimulus could be programmed within a single test session. A small fan was positioned above the sipper tube, which directed a current of air past the sipper tube

throughout each test session, minimizing the animal's detection of potential odors emanating from the taste solutions. The use of the fan was motivated by reports that the latency to initiate the first lick in brief-access trials is increased by odors from normally avoided taste stimuli (Boughter *et al.*, 2002) and decreased by odors from normally preferred taste stimuli (Rhinehart-Doty *et al.*, 1994). Using three gustometers, we could test three mice concurrently in a slightly staggered fashion and a total of 27 mice per day (nine per gustometer). Each gustometer chamber was cleaned with a weak Alconox™ solution before each test session.

All licks to a sipper tube were recorded by a high-frequency AC contact circuit designed to eliminate 'electric taste,' a phenomenon that can occur in DC contact lick detection systems if the current is not sufficiently low. The short response latency of the detection circuit (<1 ms) provided a precise measure of the onset and offset of licks. At the end of the trial, the shutter closed and a new taste stimulus was moved into position for the next trial.

The software provided with the gustometer served two functions. First, it enabled us to control the number of presentations of each taste stimulus, as well as the order and duration of each presentation. Second, it recorded the latency to the first lick of each trial and provided summary statistics for the trials associated with different taste stimulus concentrations. The number of licks, the latency to the first lick, the stimulus type and session number were also stored on a trial-by-trial basis into a sequential file, which was appended across sessions. The software program parameters were set to exclude any inter-lick interval (ILI) <70 ms so as to avoid artificially inflating lick counts with double contacts during a single tongue protrusion.

Taste stimuli

All solutions were prepared with reagent grade chemicals (Sigma-Aldrich) dissolved in deionized water and presented at room temperature.

Restricted access schedules for water and food

To encourage the mice to sample from the sipper tube during training and taste testing with QHCl and NaCl (see below), we removed their water bottles from the home cages 22.5 h before each 30 min test session so that they were thirsty when placed in the gustometer. To prevent excessive loss of body mass, the mice received a 1 h presentation of water in their home cage immediately following the session. Under this restricted water access schedule, all mice were able to maintain their body mass between 85–90% of their baseline values during training and taste testing, and showed no overt signs of distress.

To encourage sampling from the sipper tube during taste testing with sucrose, we limited the mice to 1 g of food and 2 ml of water over the 23.5 h period prior to each test session; this amounted to ~19 and 30% of the their normal daily food and water intake, respectively (J.I. Glendinning, unpublished data). In cases where sucrose testing occurred over several test sessions, a recovery day was interjected, during which food and water were available *ad-libitum* after each sucrose test session for 23.5 h. After the recovery day, each mouse was once again limited to the prescribed rations over the next 23.5 h period. Under this food and water restriction procedure, the mice: (i) maintained their body mass at >80% of their baseline values; (ii) regained 100% of their baseline body mass over each recovery day; (iii) failed to show any overt signs of distress; and (iv) exhibited vigorous concentration-dependent licking from the calorie-containing stimulus.

Sipper tube training

During days 1 and 2, the water-restricted mice were familiarized with the gustometer and trained to lick from sipper tubes (containing water). A test session began when the mouse took its first lick, and lasted 30 min. On day 1, the sipper tube was positioned in the center of the slot with the shutter permanently open. The mouse could drink as much water as possible from the single tube during the session. On day 2, the mice received more limited access to the sipper tubes—that is, the shutter was opened and a trial lasting 5–15 s (see below for details) was initiated once the mouse took its first lick from the sipper tube. At the end of a trial, the shutter was closed for 7.5 s (during which time a different sipper tube containing water was positioned in the center of the slot) and then reopened, enabling the mouse to initiate another trial of the same duration. In this manner, the mouse could initiate as many trials as possible during a test session.

During preliminary experiments, we determined that 30 min was the best test session duration. Longer trials were unnecessary because virtually all of the mice stopped initiating trials after 30 min. Shorter trials were problematic because ~25% of the mice initiated a significant number of trials (~20% of the total) during the final 10 min of the 30 min test session.

Procedure for experiment 1: determination of optimal trial duration

This experiment was designed to identify the optimal trial duration when water was the stimulus. Trial length was constant during a particular test session (e.g. 5 s), but each mouse was exposed to all three trial lengths across the three testing sessions. The testing was started on the day 2 of sipper tube training and ended on day 4. We randomized across mice the order of exposure to the three different trial lengths (5, 10, or 15 s).

For the data analysis, we examined the effect of trial length on four measures of licking performance: total licks taken, number of trials initiated, number of licks/trial and the coefficient of variation in the number of licks/trial (calculated across all trials in a test session, separately for

each mouse). We performed one-way repeated-measures ANOVAs on each response variable. We used paired *t*-tests for *post hoc* comparisons of means. Because this latter procedure involved the use of multiple paired comparisons, we corrected the α level with a sequential Bonferroni-type procedure (Benjamini and Hochberg, 1995). The α level was 0.05 (prior to correction) in this and all subsequent experiments.

Procedure for experiment 2: concentration-dependent licking to QHCl and NaCl

After determining the optimal trial duration (5 s) from experiment 1, we asked whether the water-restricted mice would exhibit a concentration-dependent suppression of licking in response to QHCl and NaCl, as has been observed in rats (Spector *et al.*, 1993; St John *et al.*, 1994). The mice were trained with water as described above. On day 2 and for the next session, the trial duration was 5 s. On day 3 (the testing day), each mouse was tested with seven concentrations of QHCl (0, 0.01, 0.03, 0.1, 0.3, 1.0 and 3.0 mM) or NaCl (0, 0.01, 0.1, 0.2, 0.3, 0.6 and 1.0 M) during a single 30 min test session. Only one taste compound was included per test session (i.e. different concentrations of either QHCl or NaCl) and only one concentration of a taste stimulus per trial. Further, to control for any concentration order effects across trials, we treated the range of concentrations of a given taste stimulus as a block and programmed our software so that it randomized (without replacement) the sequence of presentation of each stimulus concentration within each block. The mouse was permitted to initiate as many trials (and hence, blocks) as possible throughout the 30 min test session.

In this and all subsequent experiments, we standardized responses to QHCl and NaCl to responses to water for each mouse by calculating a tastant/water lick ratio. A ratio of 1.0 occurred when licks to the taste stimulus equals licks to water, indicating that the animal did not treat the taste stimulus differently from water. Ratios approaching 0.0 indicate that the taste stimulus suppressed licking relative to water. This ratio was derived to control for individual differences in local lick rate and motivational state.

For the data analysis, we conducted one-way repeated-measures ANOVAs (separately for each taste stimulus) to test for a significant effect of stimulus concentration on the tastant/water lick ratio.

Procedure for experiment 3: concentration-dependent licking to sucrose

In this experiment, we asked whether the water and food restricted mice would increase in their rate of licking in response to increasing concentrations of sucrose, as has been observed in rats (Young and Trafton, 1964; Davis, 1973; Krimm *et al.*, 1987; Smith *et al.*, 1992; Spector *et al.*, 1993, 1996). On days 1 and 2, the mice were trained with water as described above. On day 2 (and for all remaining sessions), the trial duration was 5 s. After the day 2 session, each mouse was returned to its home cage and given food and water *ad libitum* for 24 h; this period served as a recovery day. On day 3, each mouse was reweighed and presented with its restricted food (1 g) and water (2 ml) ration as described above. On day 4 (testing day), each mouse was reweighed, placed in the gustometer and tested with a range of sucrose concentrations (0, 0.03, 0.01, 0.2, 0.3, 0.6 and 1.0 M) over the 30 min test session.

The tastant/water lick ratio was not useful for standardizing responses to sucrose solutions, because the licking responses of water-restricted mice to water alone were almost as vigorous as those to high concentrations of sucrose. In its place, we calculated a different type of score, called the standardized lick ratio. We accomplished this by measuring the local lick rate (see below for an explanation) based on each mouse's licking responses during the test session on training day 1 (see above). Then, we multiplied the local lick rate (expressed as licks/s) by a scaling factor of five, resulting in an estimate of the maximal number of licks that the mouse could generate if it licked continuously during the 5 s trial. Finally, the average number of licks/trial directed at each sucrose concentration was divided by the maximal potential lick rate per trial, yielding the standardized lick ratio. A standardized lick ratio approaching 0.0 indicates that the sucrose concentration elicited minimal licking, whereas a value of 1.0 indicates that the sucrose concentration elicited maximal licking. Although this ratio does not necessarily control for variation in the motivational state arising from differential responses to the food and water restriction schedule, it does control for individual differences in local lick rate.

We calculated the local lick rate for each mouse as follows. First, the customized software calculated the mean ILI for each mouse on training day 1. The ILI is defined as the duration between the onset of two consecutive licks. We included only those ILIs < 200 ms because longer values are thought to reflect pauses between bursts of licking (Corbit and Luschei, 1969; Halpern, 1977; Horowitz *et al.*, 1977; Weijnen, 1977; Smith *et al.*, 1980; Davis, 1989; Spector *et al.*, 1998). The local lick rate is the reciprocal of the ILI measure.

For the data analysis, we used one-way repeated-measures ANOVAs to test for significant effects of sucrose concentration on the standardized lick ratio.

Procedure for experiment 4: test for changes in concentration-dependent licking responses across test sessions

In experiments 2 and 3, we obtained concentration–response functions for QHCl, NaCl and sucrose during a single 30 min test session. In this experiment, we examined the extent to which the concentration–response curves would change as trials from additional sessions were included in the analysis. On days 1 and 2, the mice were trained with

water as described above. Then, each mouse was tested for its responsiveness to a range of concentrations of QHCl (0, 0.006, 0.01, 0.03, 0.1, 0.3 and 1.0 mM), NaCl (0, 0.01, 0.1, 0.2, 0.3, 0.6 and 1.0 M) or sucrose (0, 0.03, 0.01, 0.2, 0.3, 0.6 and 1.0 M). During each test session, mice received all concentrations of a single taste compound according to a randomized trial schedule as described above. Each mouse was tested over three sessions with only one taste compound (e.g. QHCl).

It took 3 days to complete the test sessions with QHCl or NaCl. Each test session was preceded by 22.5 h of water deprivation and followed by 1 h of water supplementation. It took twice as long to complete the tests with sucrose because a ~24 h recovery period was interjected between each sucrose test session.

We evaluated the effect of including trials from additional sessions with two-way repeated-measures ANOVAs (separately for each taste stimulus). We determined whether the concentration–response curves changed as additional test sessions were included (both taste stimulus concentration and test session were treated as within factors). Second, we examined the SD of the concentration–response functions across mice to determine whether they became more or less variable as additional test sessions were included.

Procedure for experiment 5: application of the phenotypic screening procedure and collection of normative data

In this final experiment, we designed a phenotypic screening procedure for assessing oromotor and taste function in individual mice, based on results from experiments 1–4. Our general approach involved running a large sample of wild-type C57BL/6J mice through the screen and then using the results from this large population of mice to derive a normative database, against which one could subsequently compare the responses of the progeny of mutagenized (or genetically altered) mice. The timetable for the phenotypic screen, together with the associated deprivation procedures, is provided in Table 1. In summary, the mice received sipper tube training on days 2–3; QHCl testing on days 4, 5 and 9; NaCl testing on day 10; and sucrose testing on day 12. We deliberately configured the timetable to accommodate mutagenesis centers (or individual laboratories) that are in operation only 5 days a week (i.e. Monday to Friday). The rationale for using three test sessions for QHCl and one test session each for NaCl and sucrose, is provided in the account of experiment 4. We used the same range of concentrations of each taste stimulus as in experiment 4.

The screen for oromotor function was derived from the licking responses of mice to water during the first training session. First, we calculated three measures of licking for each mouse: (i) the mean of the population of ILIs <200 ms provided a measure of licking rate; (ii) the SD of the population of ILIs <200 ms provided a measure of licking consistency; and (iii) the total number of licks across the test session provided a measure of overall licking activity.

Table 1 Timetable of the phenotypic screen for taste function

Day	Activity	Food and water access prior to testing
1	water deprivation begins	food and water *ad libitum*
2	spout training	22.5 h water deprivation
3	shutter training	22.5 h water deprivation
4	QHCl testing, session 1	22.5 h water deprivation
5	QHCl testing, session 2	22.5 h water deprivation
6	none	food and water *ad libitum*
7	none	food and water *ad libitum*
8	water deprivation begins	food and water *ad libitum*
9	QHCl testing, session 3	22.5 h water deprivation
10	NaCl testing	22.5 h water deprivation
11	recovery day	food and water *ad libitum*
12	sucrose testing	23.5 h food/water restriction

Second, we determined whether there was a significant effect of gender on any of the licking parameters, using a one-way ANOVA. Then, we compiled a frequency distribution for each licking parameter to help identify outliers.

The screen for taste function was derived from the concentration–response curves for each taste stimulus. The concentration–response curves for QHCl were based on responses averaged across trials from three test sessions and those for NaCl and sucrose were based on responses averaged across trials from one test session. Two-way repeated-measures ANOVAs were performed to test for a significant effect of stimulus concentration and gender on the tastant/water lick ratio for QHCl and NaCl, or the standardized lick ratio for sucrose. Next, we calculated the mean response of each mouse to the two lowest concentrations of each taste stimulus, the two intermediate concentrations and the two highest concentrations. Then, we constructed three frequency distributions based on these averaged scores, one for each range of concentrations. Finally, we calculated the mean and SD for the respective distributions.

We chose values that were ±3 SD from the mean as the outlier criteria for each of the distributions described above, but investigators are free to choose more or less conservative cut-offs. We determined separate outlier criteria for males and females if the ANOVA described above detected a significant effect of gender. We reasoned that these normative distributions would help identify mice with taste anomalies. Any mouse with a response that falls outside the outlier criteria of any of these distributions would be flagged as having a potentially interesting taste anomaly.

Finally, we asked whether odors emitted by the taste stimuli influenced the appetitive phase of the licking responses (i.e. the latency to initiate the first lick during a trial). We randomly sampled 21 mice from the normative database (12 females and nine males) and then compared their latencies

to initiate licking from the various stimuli. We conducted paired comparisons with a nonparametric procedure (the Wilcoxon matched-pairs signed-rank test), because the latency data were not normally distributed. To control for the use of multiple paired comparisons, we used a sequential Bonferroni-type correction (Benjamini and Hochberg, 1995).

Results

Results for experiment 1: determination of optimal trial duration

Trial length (5, 10 or 15 s) affected some but not all features of licking performance with water alone. It did not influence the total number of licks taken during the test session [$F(2,28) = 0.06$, $P > 0.05$]. The mice took an average of 600–700 licks per test session, irrespective of trial length (Figure 1a). Trial length did influence the total number of trials initiated during a test session [$F(2,28) = 8.30$, $P < 0.05$]. The mice initiated significantly more trials when the trial length was 5 s than when it was 10 or 15 s (Figure 1b). Trial length also influenced the mean number of licks taken per trial [$F(2,28) = 11.6$, $P < 0.05$; Figure 1c] and the coefficient of variation (CV) of the number of licks taken per trial [calculated across all mice; $F(2,28) = 18.3$, $P < 0.05$; Figure 1d]. Even though the mice took significantly fewer licks during the 5 s trials, they tended to lick more consistently throughout the 5 s periods. This latter finding reflects the fact that mice paused frequently during the 10 and 15 s trials. We decided to use a 5 s trial duration in all subsequent experiments, because it caused the mice to initiate the greatest number of trials and to lick most consistently.

Results for experiment 2: concentration-dependent licking to QHCl and NaCl

The licking response to QHCl and NaCl solutions became progressively more inhibited (i.e. the tastant/water lick ratio decreased) as the concentration of each taste stimulus was increased [QHCl, $F(5,65) = 71.0$, $P < 0.05$; NaCl, $F(5,65) = 80.7$, $P < 0.05$; Figure 2a,b]. At the higher concentrations, licking was almost completely inhibited. These results establish that the experimental procedures developed for QHCl and NaCl (i.e. the specific range of concentrations of each taste stimulus, the 5 s trial duration and the restricted water access schedule) produce robust concentration–response curves.

Results for experiment 3: concentration-dependent licking to sucrose

The licking responses to sucrose became progressively more vigorous (i.e. the standardized lick ratio became greater) as the concentration of the sugar solution was increased [$F(5,65) = 46.6$, $P < 0.05$; Figure 2c]. At the highest sucrose concentration, the standardized lick rate approached 0.65,

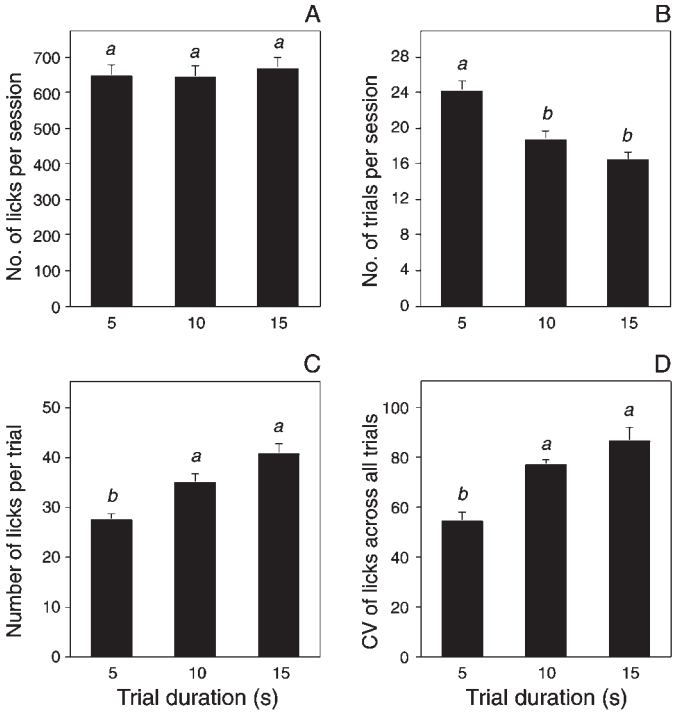

Figure 1 Effect of varying trial length on **(a)** the total number of licks per session, **(b)** the number of trials per session, **(c)** the number of licks per trial and **(d)** the coefficient of variation (CV) in mean number of licks per trial. We present mean ± SE ($n = 14$). Different letters above each bar (*a* or *b*) indicate which mean values differ significantly from one another (within each panel), according to multiple paired *t*-tests ($\alpha = 0.05$). The α level was corrected for the use of multiple paired *t*-tests by a sequential Bonferroni-type procedure (see text for details).

indicating that, on average, the mice licked at ~65% of their maximal rate. These results show that the experimental procedures developed for sucrose (i.e. the specific range of concentrations, the 5 s trial duration and the water and food restriction schedule) generate a broad range of concentration-dependent licking responses.

Results for Experiment 4: Test for changes in concentration-dependent licking responses across test sessions

For QHCl, the mean values of the tastant/water lick ratios did not change as trials from additional test sessions were included in the analysis [$F(2,30) = 0.08$, $P > 0.05$]. Although there was a significant effect of stimulus concentration [$F(5,75) = 103.0$, $P < 0.05$], there was no significant interaction between test session and stimulus concentration [$F(10,150) = 1.33$, $P > 0.05$]. There was a marked reduction, however, in the variance of the tastant/water lick ratios (both within and between mice) as trials from additional test sessions were included, particularly at the lower concentrations. This is apparent in Figure 3 (top panels): the concentration–response curves became more tightly grouped and the SD of the means diminished as trials from additional test sessions were included in the analysis. These

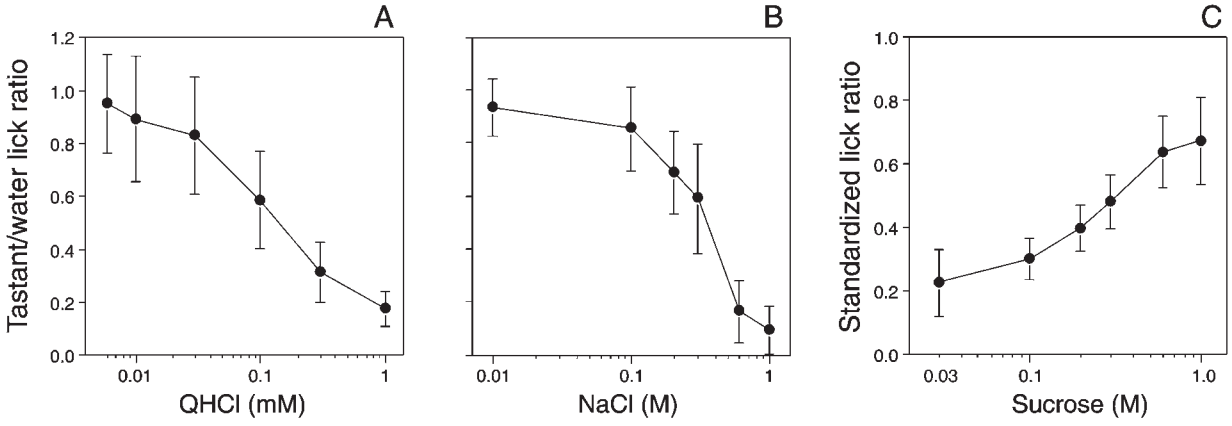

Figure 2 Concentration–response curves for **(a)** QHCl, **(b)** NaCl and **(c)** sucrose (mean ± SD). The response variable for QHCl and NaCl is the tastant/water lick ratio and for sucrose the standardized lick ratio; $n = 16$ mice per panel, with approximately equal numbers of males and females.

results led us to conclude that three test sessions would be sufficient for QHCl testing.

For NaCl, the mean values of the tastant/water lick ratios also did not change as trials from additional test sessions were included in the analysis [$F(2,26) = 0.58$, $P > 0.05$]. As with QHCl, there was a significant effect of stimulus concentration [$F(5,75) = 107.5$, $P < 0.05$], but no significant interaction between test session and stimulus concentration [$F(10,130) = 0.91$, $P > 0.05$]. However, unlike QHCl, the group data for NaCl did not become less variable as trials from different test sessions were included. Visual inspection of Figure 3 (middle panels) shows that, with one exception, the concentration–response curves were all near 1.0 at the low concentrations and then plummeted to almost zero at the higher concentrations (i.e. between 100 and 600 mM NaCl). This basic profile was observed regardless of the number of test sessions included in the analysis. These findings led us to conclude that one test session was sufficient for NaCl testing.

The response to sucrose was unusual because the mean values of the tastant/water lick ratios actually diminished slightly as trials from additional test sessions were included in the analysis. Despite a nonsignificant effect of including additional test sessions [$F(2,28) = 1.05$, $P > 0.05$] and a significant effect of stimulus concentration [$F(5,75) = 206.9$, $P < 0.05$], there was a significant interaction between these two main effects [$F(10,140) = 2.62$, P 0.05]. The significant interaction term reveals a concentration-dependent effect of including additional test sessions, which stemmed from the mice licking the intermediate concentrations of sucrose slightly more during the first testing day (Figure 3, bottom left panel). Although there was a statistically significant change in sucrose responding with additional test sessions, the tight grouping of the concentration–response curves, coupled with their uniformly rising slopes, led us to conclude that a single test session was sufficient for sucrose testing (Figure 3, bottom panels).

Results for experiment 5: development of the phenotypic screening procedure

We initially evaluated the mice (48 females and 50 males) for oromotor function, using the spontaneous licking responses to water during the first training day. Distributions of the ILI values were composed for each mouse and the means and SDs were subjected to ANOVAs. There was a significant effect of gender on both the SD of the ILI [$F(1,97) = 7.52$, $P < 0.05$] and the total number of licks generated during the test session [$F(1,97) = 8.33$, $P < 0.05$], but not on the mean ILI value [$F(1,97) = 0.11$, $P > 0.05$]. These gender effects reflect the fact that the males exhibited more licks, while the females exhibited more variable ILIs (Table 2). We present the means and the outlier criteria (i.e. 3 SD from the mean) for each licking parameter in Table 2 and in Figure 4. Any mouse that generated a licking parameter that was greater or less than any of the outlier criteria would be presumed to have an oromotor anomaly.

There was no concentration-dependent change in the latency to initiate the first lick for any of the taste stimuli (Figure 5). For sucrose, there was a tendency for the latency to decrease with increasing concentrations, but this effect was not significant. For NaCl, there was a significant increase in the latency for all but one of the intermediate NaCl concentrations, but there was no evidence that the latency increased in a concentration-dependent manner. For QHCl, despite considerable individual variation, there was no evidence of a concentration-dependent change in the latency to initiate licking. The median latency to initiate licking from the QHCl solutions, however, was considerably longer than that for NaCl or sucrose solutions. This latter finding can be explained in part by the aversive hedonic attributes of the QHCl solutions. Taken together, the lack of concentration dependence to the licking responses indicates that they were not influenced significantly by odor cues emitted by the taste stimuli.

Both male and female mice generated orderly concentration–response functions for the three taste compounds

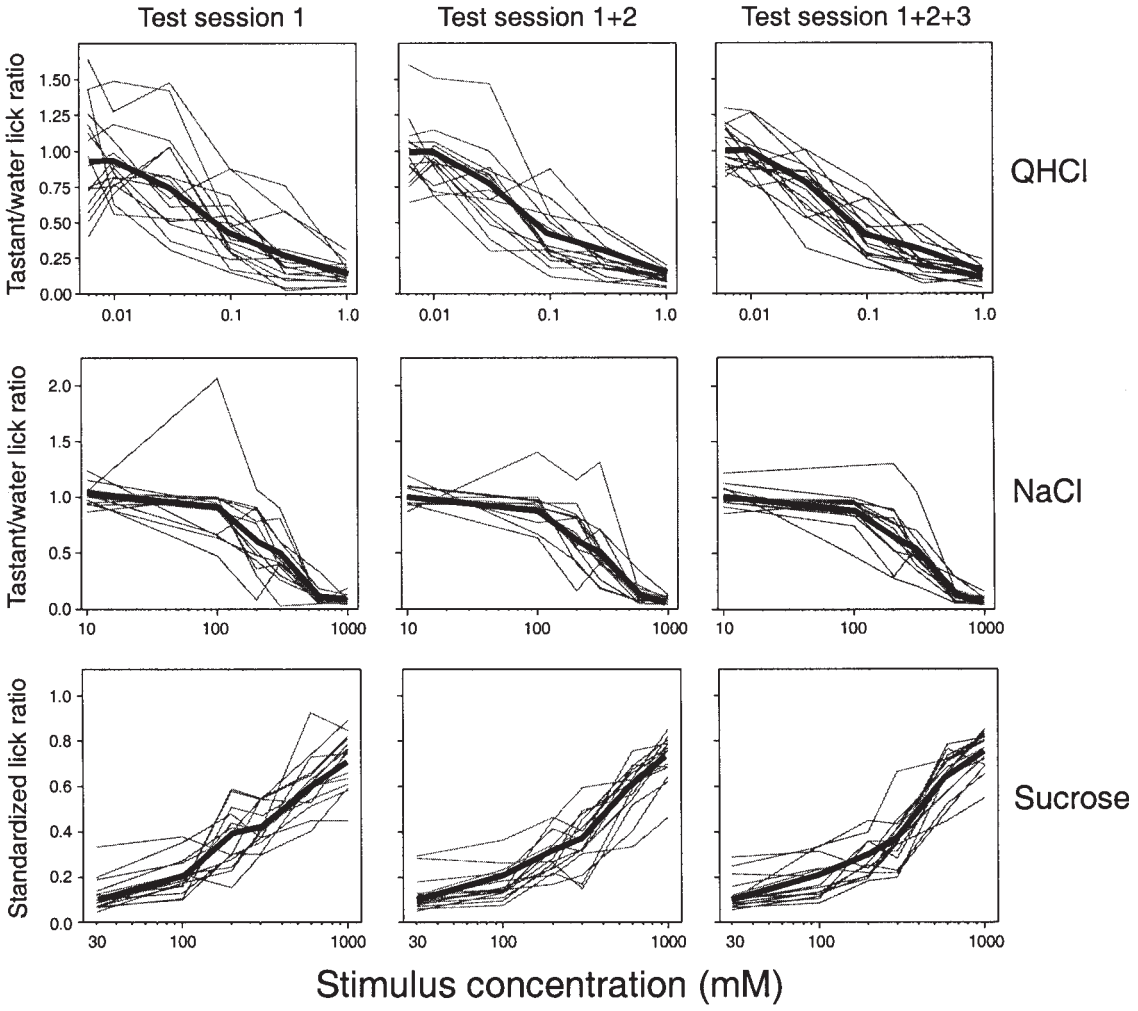

Figure 3 Licking responses to different concentrations of QHCl (top panels), NaCl (middle panels) and sucrose (bottom panels) across three successive testing sessions. In each panel, the licking response of each mouse (n = 14–15 mice per taste stimulus) is represented by the thin gray lines, whereas the mean response of all mice is represented by a thick black line. We present results from trials that occurred during test session 1, test sessions 1 + 2 and test sessions 1 + 2 + 3. The tastant/water lick ratio is the response variable for QHCl and NaCl and the standardized lick ratio is the response variable for sucrose. See text for statistical analysis of these data.

(Figure 6a–c). The concentration–response curves for QHCl and NaCl decreased monotonically from a mean tastant/water lick ratio of ~0.95 to 0.20. The salient difference between these two taste stimuli was that the QHCl curves decreased more gradually than did the NaCl curves (i.e. over a span of 2 versus 1 $\log_{10}$ concentration units, respectively). The concentration–response curves for sucrose increased monotonically over one order of magnitude in concentration from a mean standardized lick ratio of ~0.20 to 0.70. The two-way ANOVA showed that for QHCl and sucrose, the main effect of concentration [$F(5,485) < 232$, $P < 0.05$] was significant, but the main effect of gender [$F(1,97) < 1.38$, $P > 0.05$] and the interaction [$F(5,485)$ 1.43, $P > 0.05$] were not. For NaCl, the main effects of concentration [$F(5,485) = 409.2$, $P < 0.05$] and gender [$F(1,97) = 3.95$, $P = 0.05$] were both significant, but the interaction was not [$F(5,485) = 0.23$, $P > 0.05$]. The latter finding reveals that the

Table 2 Critical values of the screen for oromotor function

Licking parameter	Gender	Mean	SD	Lower outlier criterion	Upper outlier criterion
Interlick interval (ms)	male	122.5	4.6	108.7	136.4
	female	122.1	4.0	110.2	134.0
	both	122.3	4.3	109.5	135.1
SD of interlick interval	male	16.7	2.5	9.2	24.2
	female	18.3	3.0	9.3	27.4
	both	17.5	2.9	8.9	26.2
Total no. of licks	male	487.5	138.7	71.5	903.4
	female	408.1	119.4	50.6	765.5
	both	446.6	134.4	43.5	849.6

The outlier criteria indicate values that are 3 SD above and below the mean. n = 50 male and 48 female mice.

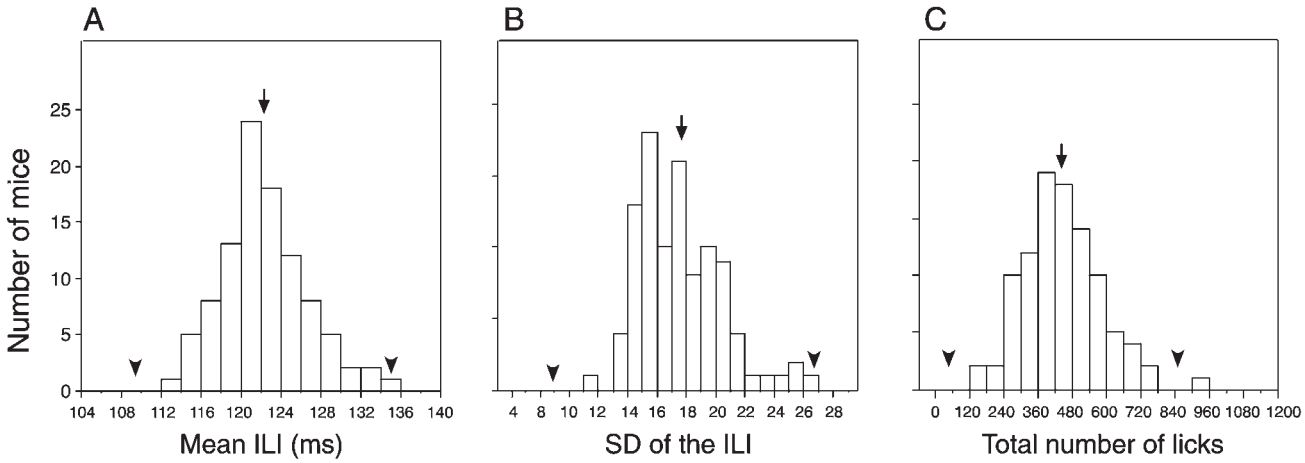

Figure 4 Method for evaluating oromotor competence of mice. We present frequency distributions of **(a)** the inter-lick interval (ILI), **(b)** the SD of the ILI values and **(c)** the total number of licks taken during the test session. We indicate the mean of each distribution with an arrow and the outlier criteria for each distribution (i.e. the values 3 SD above and/or below the mean) with an arrowhead. Any mouse that generates a licking parameter that is greater or less than any of the outlier criteria would be presumed to have an oromotor anomaly; $n = 98$ mice.

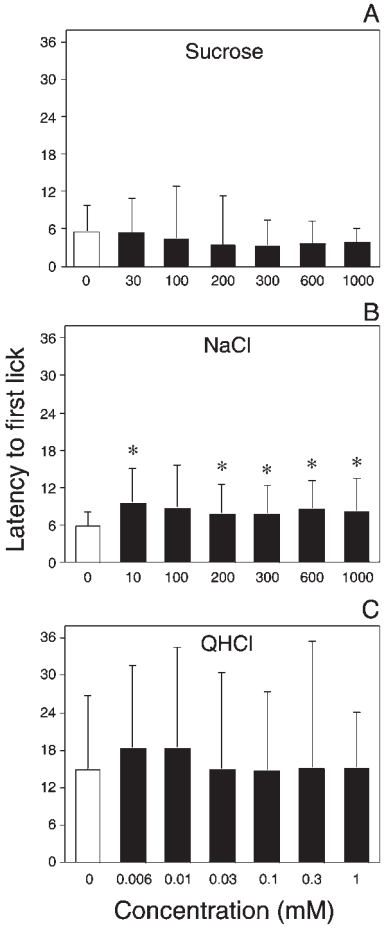

Figure 5 Latency to the first lick during trials with different concentrations of **(a)** sucrose, **(b)** NaCl and **(c)** QHCl (median ± median absolute deviation). Within each panel, we compare the median latency to first lick for each stimulus concentration with that for water, using Wilcoxon matched-pairs signed-rank tests. We corrected for the use of multiple paired tests with a sequential Bonferroni-type procedure (Benjamini and Hochberg, 1995). *$P < 0.05$.

females (on average) licked slightly more than did the males from all concentrations of NaCl relative to water. It is unlikely that this finding can be simply explained by differences in mass, because the males weighed significantly more than the females (on day 1 of testing; see Table 1), despite being age-matched [mean mass ± SD = 22.8 ± 2.2 and 18.0 ± 1.1, respectively; $t(97) = 13.7$, $P < 0.05$, unpaired t-test].

Figure 7 contains frequency distributions for the low, intermediate and high concentration ranges of QHCl, NaCl and sucrose, collapsed across gender. For QHCl and NaCl, the mean and SD of the frequency distributions decreased markedly with concentration (Table 3). For sucrose, the means of each distribution increased with concentration, but the SDs remained relatively constant (Table 3).

Investigators can use the values in Table 3 as a normative database against which to compare the responses of the progeny of mutagenized (or genetically altered) mice. Accordingly, mice that are hypogeusic to QHCl or NaCl should generate tastant/water lick ratios that surpass the upper outlier criteria and mice that are hypogeusic to sucrose should generate standardized lick ratios that are less than the lower outlier criteria. In contrast, mice that are hypergeusic to QHCl or NaCl should generate tastant/water lick ratios that are less than the lower outlier criteria and mice that are hypergeusic to sucrose should generate standardized lick ratios that surpass the upper outlier criteria.

We selected 3 SD from the mean as the outlier criterion for identifying subjects with aberrant taste function because it has been used successfully in other phenotypic screening procedures with mice (Nolan *et al.*, 1997; Sayah *et al.*, 2000). It is important to note, however, that an individual investigator could set more relaxed or stringent outlier criteria (e.g. 2 or 4 SD, respectively) based on the information provided in Table 3.

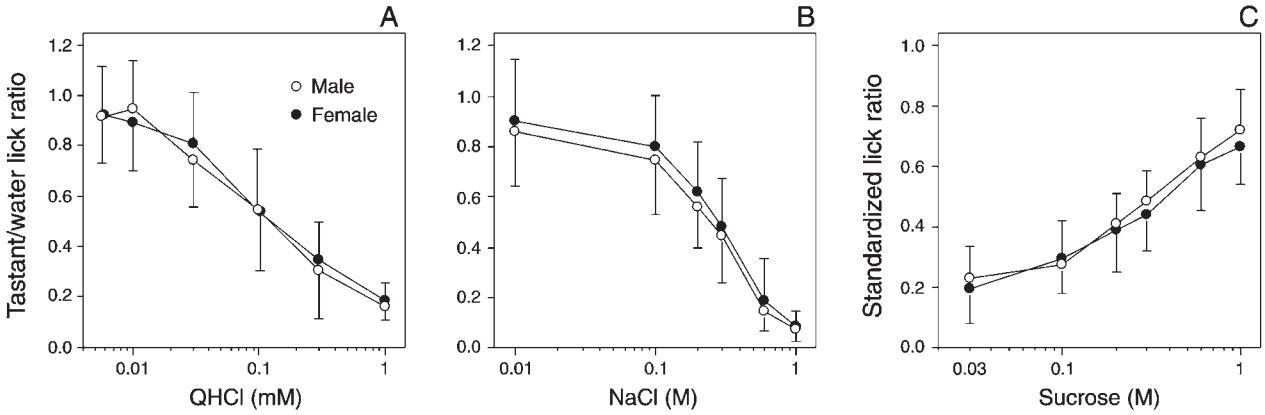

Figure 6 Concentration–response curves for **(a)** QHCl, **(b)** NaCl and **(c)** sucrose for the mice in the normative database (mean ± SD). Males and females are plotted separately. The response variable for QHCl and NaCl is the tastant/water lick ratio and for sucrose is the standardized lick ratio; $n = 48$ female and 50 male mice.

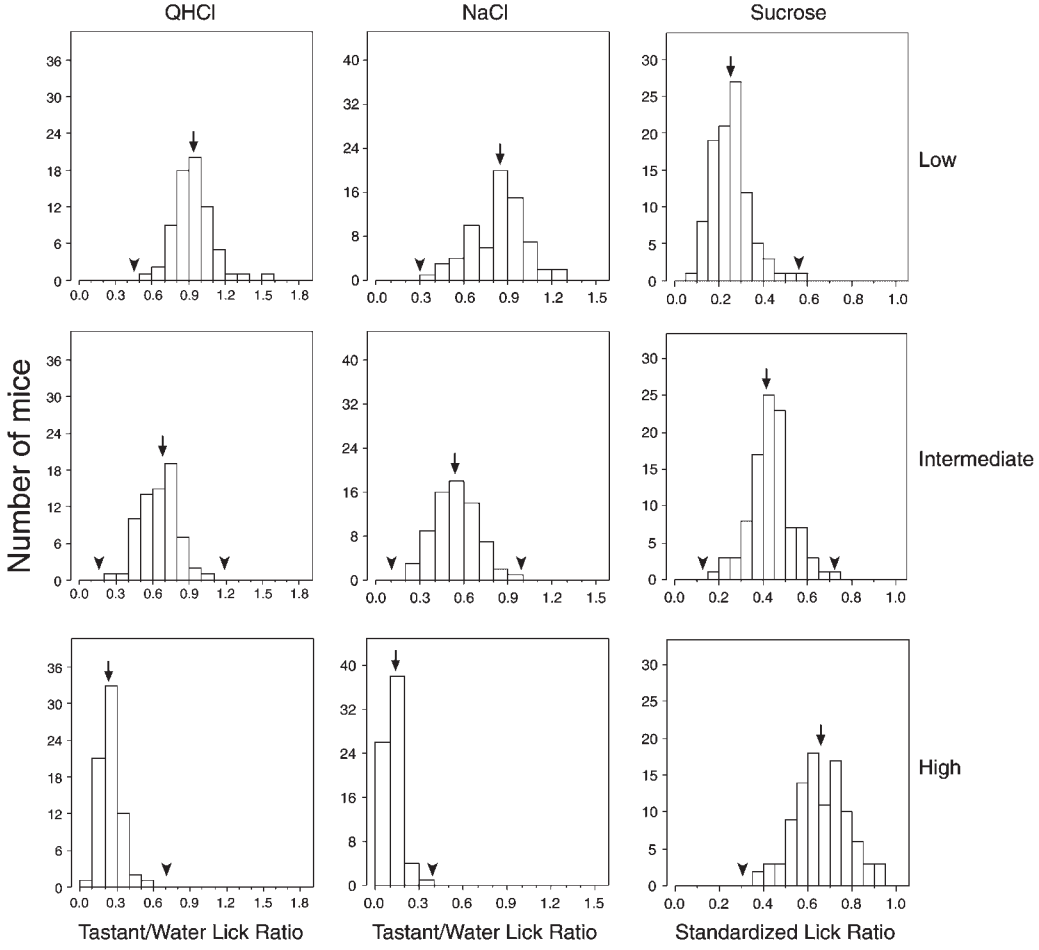

Figure 7 Method for evaluating taste function in individual mice. We present frequency distributions of the licking responses to low, intermediate and high concentrations of QHCl (left column of panels), NaCl (middle column of panels) and sucrose (right column of panels), based on 98 mice. We indicate the mean of each distribution with an arrow and the outlier criteria for each distribution (i.e. the values 3 SD above and/or below the mean) with an arrowhead. The low, medium and high concentration ranges of each taste stimulus correspond to the lowest two, middle two and highest two concentrations that were tested (see text for details). For the low concentration range, we provide an outlier criterion to identify mice with hypergeusia (i.e. a response below the outlier criterion for QHCl or NaCl, or above that for sucrose). For the high concentration range, we provide an outlier criterion to identify mice with hypogeusia (i.e. a response above the outlier criterion for QHCl or NaCl, or below that for sucrose). For the intermediate concentration range, we provide outlier criteria to identify mice with hyper- or hypogeusia. For instance, a mouse that is hypogeusic to QHCl should produce a tastant/water lick ratio that is above the outlier criteria for both the intermediate and high concentration ranges.

Table 3 Critical values of the screen for taste function, based on the distributions of the tastant/water or standardized lick ratios presented in Figure 7

Taste stimulus	Lick ratio	Gender	Range of concentrations	Mean	SD	Lower outlier criterion	Upper outlier criterion
QHCl	tastant/water	male	low	0.93	0.16	0.46	–
			medium	0.64	0.17	0.12	1.16
			high	0.23	0.08	–	0.48
		female	low	0.91	0.16	0.44	–
			medium	0.69	0.20	0.10	1.28
			high	0.26	0.09	–	0.54
		both	low	0.92	0.16	0.45	–
			medium	0.66	0.17	0.15	1.17
			high	0.24	0.09	–	0.47
NaCl	tastant/water	male	low	0.80	0.17	0.29	–
			medium	0.50	0.15	0.05	0.95
			high	0.11	0.06		0.29
		female	low	0.85	0.19	0.28	–
			medium	0.55	0.15	0.10	1.00
			high	0.14	0.11	–	0.47
		both	low	0.83	0.18	0.28	–
			medium	0.53	0.15	0.07	0.98
			high	0.12	0.09	–	0.38
Sucrose	standardized	male	low	0.24	0.10	–	0.54
			medium	0.43	0.09	0.16	0.69
			high	0.69	0.11	0.35	–
		female	low	0.26	0.09	–	0.52
			medium	0.44	0.11	0.11	0.77
			high	0.61	0.13	0.22	–
		both	low	0.25	0.09	–	0.52
			medium	0.43	0.10	0.13	0.73
			high	0.66	0.12	0.30	–

The concentration ranges of each taste stimulus are represented by the two lowest, the two middle and the two highest concentrations tested (see text for details). The outlier criteria are 3 SD above and/or below the mean. $n = 50$ male and 48 female mice.

Discussion

We have demonstrated that the brief-access taste test has great potential to be an effective and efficient way to screen individual mice for aberrant taste function. The screening procedure presented here can generate an orderly concentration–response curve for NaCl or sucrose in a single 30 min test session and for QHCl over three 30 min test sessions. Licking responses from various portions of the dynamic range of the concentration–response functions can then be compared on a mouse-by-mouse basis with our normative database to detect gross or subtle alterations in taste responsiveness. The data obtained during the first sipper training session can be used to identify any mouse with a potential oromotor dysfunction (e.g. stemming from the mutagenesis procedure), enhancing the power of the screen to dissociate gustatory from motor influences in licking responses. Throughout the development of the screening protocol, we sought to balance two conflicting demands: the need to maximize the screen's sensitivity and minimize its duration. Toward this end, we determined the shortest possible trial duration and the fewest number of test sessions necessary to yield reliable estimates of responsiveness to each taste stimulus. The food and water restriction protocols clearly motivated the mice to lick in a concentration-dependent manner during each test session, but did not lead to excessive loss of body mass or overt signs of distress.

Several features of the screen increase the likelihood that the licking responses are mediated principally by taste and are not confounded by nongustatory effects of the chemical stimuli. First, a stream of air was passed over the sipper tube to help disperse any odors released by the taste stimuli. This procedure appears to have been effective because there was no systematic change with concentration in the latency to initiate licking from any of the taste stimuli. Second, the duration of each trial was limited to 5 s to reduce the potential for postingestive feedback. Third, the sequence of stimulus presentation was randomized within each consecutive block to minimize systematic order and contrast effects.

The oromotor screen is not only useful for reducing the number of false negatives and false positives in the taste screen, but is also useful in its own right. Licking is one of

the most robust and reliable behaviors elicited by rodents and the oromotor screen could help investigators identify specific genes that mediate the development and maintenance of this motor system. For example, the time period between successive lick onsets can be divided into two classes. The first class is the ILI, which is thought to be governed by a central pattern generator (Halpern, 1977; Weijnen, 1977; Travers *et al.*, 1997). The ILIs are short in duration and form a relatively tightly clumped and symmetrical distribution around the mean value, representing the fundamental period of the central pattern generator output. The second class represents time periods between licks that are longer in duration and fall well outside the primary ILI distribution. These are thought to reflect pauses between bursts of licking. Under consistent testing conditions, a mouse's ILI is remarkably stable, although this parameter can vary across inbred strains (Horowitz *et al.*, 1977). Our oromotor screen would be ideal for evaluating large numbers of mice with potential mutations for abnormalities in ILI. Any mouse with an ILI distribution that falls outside the range of the normative criteria could be considered an outlier and subjected to more detailed testing.

Owing to the automated nature of our procedure, a single laboratory technician with limited experience should be able to execute the screen competently. At the start of the day, the technician would prepare the taste stimulus solutions and fill the reservoirs of the gustometer. A user-friendly control computer program (provided by the manufacturer) would direct the testing (or training) session, separately for each gustometer. Based on our experience, the same technician should be able to test mice using at least five gustometers at a time in a slightly staggered fashion. At the end of the test session, an analysis program (provided by the manufacturer) would provide a printout detailing perform- ance and electronically store this information for any additional analyses that may be desired. These values could be compared to the distributions presented herein in the identification of outliers. Analysis of quinine performance would require loading the file containing the concatenated session data into a separate analysis program to calculate the necessary means for comparison with the normative distribution.

We estimate that a mutagenesis center (or laboratory) with 10 gustometers could screen a total of 2340 mice per year. This estimate assumes that nine mice are tested per day in each of the gustometers (=90 mice/day) and that testing occurs 5 days per week throughout the year, as described in Table 1. The screening protocol was designed to accommodate the standard work schedule of laboratory personnel and thus no manipulations are conducted on weekends. If testing could be conducted 7 days a week, then >3600 mice could be tested per year, but it is unclear how such a modification in the protocol schedule would affect performance. Accordingly, it would be prudent to derive a new and more appropriate normative database if such a change in the procedure was instituted. For researchers interested only in screening for oromotor abnormalities, we estimate that a total of 16 425 mice could be tested per year.

The ability of our screening protocol to generate complete concentration–response curves for three taste stimuli in 12 days (7 days of training and testing) represents a significant methodological advance over more common procedures. For example, it would take 42 days to test the same range of concentrations of each taste stimulus using a 48 h two-bottle preference test and 84 days if a 96 h test was administered. A 12 day testing period compares favorably with some, but not all, screening procedures currently in use at mutagenesis centers (Nolan *et al.*, 1997; Sayah *et al.*, 2000). For example, whereas some screening procedures (e.g. tests for abnormal circadian wheel running activity) take several weeks to complete, many others (e.g. tests for anomalous sensorimotor gating, learning and memory, nociception and locomotor activity) can each be completed within a 30 min period. We are currently developing a more rapid taste screen that may take as few as 5 days to complete, but we do not expect to be able to shorten the screen further than that and the information that this more primary screen would provide would be much more interpretively limited. We suspect that our situation is not unique. It may be difficult to develop extremely high-throughput (i.e. 30 min) screens for many types of behavior because (i) it often takes several training sessions to habituate an animal to a testing procedure and (ii) individuals often must be tested more than once in an experimental procedure to obtain a reliable estimate of their typical response.

Although the screening procedure described herein has many advantageous features, it also has some interpretive limitations. First and foremost is the fact that mice must be tested under restricted water and/or food conditions. This is necessary to motivate the mice to sample the taste solutions and to encourage them to lick in a concentration-dependent manner. If mice have different physiological responses to the deprivation conditions, then their motivation to perform in the task may differ. For example, some mice may become 'thirstier' than others as a result of the deprivation procedure and thus would be expected to lick more vigorously from all sipper tubes (e.g. those containing low and high concentrations of NaCl alike). Our tastant/water lick ratio controls for this possible scenario, however, by dividing the number of licks to each tastant solution by the number of licks to water.

The situation with sucrose testing is more vexing. In a preliminary experiment, we offered seven nondeprived C57BL/6J mice 0.3 M sucrose in the gustometer. They all ignored this taste stimulus after taking a few licks. This finding was unexpected because the same stimulus elicits vigorous licking in nondeprived laboratory rats (Krimm *et al*., 1987; Spector *et al*. 1998). Based on the lack of response of the non-deprived mice to 0.3 M sucrose, we developed the restricted food/water access schedule. This schedule

motivated the mice to lick vigorously and in a concentration-dependent manner from sucrose. It is possible that variation in the slope and asymptote of the sucrose concentration–response curve could reflect individual differences in the level of hunger and thirst produced by the food/water restriction schedule. Although the standardized lick ratio controls for individual variation in licking rate, it does not control for individual variation in physiological state. Thus, this caveat must be considered in the evaluation of outliers on the sucrose test.

It is also important to note that the appropriateness of our normative distributions as a comparison database depends on strict adherence to the designed protocol set forth herein. It may be convenient for investigators to customize the procedures detailed here for their own purposes. However, this should be done with the knowledge that the C57BL/6J normative database may no longer apply. There are also other limitations on the interpretation of outlying phenotypes. Once a mouse with a taste anomaly is identified, further studies will be needed to determine whether the anomaly is heritable and how it manifests itself. For instance, the anomalous behavior could be due to genetically induced alterations in the mouse's hedonic response to the taste stimulus, its sensitivity to the taste stimulus and/or the perceived quality of the taste stimulus. Furthermore, the origin of such altered taste function could reside anywhere along the gustatory pathway. We are currently developing systematic procedures for addressing some of these issues (Eylam and Spector, 2002).

In conclusion, our screening protocol should be able to identify mice with either enhanced or diminished gustatory responsiveness to a variety of taste stimuli, caveats notwithstanding. We have focused on C57BL/6J mice and three taste stimuli in this study. Elsewhere (unpublished data), we have found that the testing procedures described herein generate robust concentration–response curves with other taste stimuli (e.g. denatonium benzoate, cycloheximide and an artificial sweetener called SC45647) and with other inbred strains of mouse (e.g. SWR/J, DBA/2J, C3HeB/HeJ and 129/J). The high-throughput and automated nature of this procedure makes it useful for testing large numbers of mice in a relatively brief amount of time.

Acknowledgements

We thank Sally Guerrasio, Joon Young (Mimi) Park and Ji Yong Kong for help collecting the behavioral data. This project was supported in part by research grant No. 5 R01 DC004475 from the National Institute on Deafness and Other Communication Disorders, National Institutes of Health (to J.I.G. and A.C.S.). All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Columbia University.

References

Bachmanov, A.A., Reed, D.R., Ninomiya, Y., Inoue, M., Tordoff, M.G., Price, R.A. and **Beauchamp, G.K.** (1997) *Sucrose consumption in mice: major influence of two genetic loci affecting peripheral sensory responses*. Mamm. Genome, 8, 545–548.

Bachmanov, A.A., Tordoff, M.G. and **Beauchamp, G.K.** (1998) *Voluntary sodium chloride consumption by mice: differences among five inbred strains*. Behav. Genet., 28, 117–124.

Bargmann, C.I. (1993) *Genetic and cellular analysis of behavior in* C. elegans. Annu. Rev. Neurosci., 16, 47–71.

Battey, J., Jordan, E., Cox, D. and **Dove, W.** (1999) *An action plan for mouse genomics*. Nature Genet., 21, 73–75.

Benjamini, Y. and **Hochberg, Y.** (1995) *Controlling the false discovery rate: a practical and powerful approach to multiple testing*. J. R. Stat. Soc., Ser. B, 57, 289–300.

Blizard, D.A., Kotlus, B. and **Frank, M.E.** (1999) *Quantitative trait loci associated with short-term intake of sucrose, saccharin and quinine solutions in laboratory mice*. Chem. Senses, 24, 373–385.

Boughter, J.D., Harder, D.B., Capeless, C.G. and **Whitney, G.** (1992) *Polygenic determination of quinine aversion among mice*. Chem. Senses, 17, 427–434.

Boughter, J.D., St John, S.J., Noel, D.T., Ndubuizu, O. and **Smith, D.V.** (2002) *A brief-access test for bitter taste in mice*. Chem. Senses, 27, 133–142.

Breslin, P.A.S., Spector, A.C. and **Grill, H.J.** (1993) *Chorda tympani section decreases the cation specificity of depletion-induced sodium appetite in rats*. Am. J. Physiol. Regul. Integr. Comp. Physiol., 264, R319–R323.

Corbit, J.D. and **Luschei, E.S.** (1969) *Invariance of the rat's rate of drinking*. J. Comp. Physiol. Psychol., 69, 119–125.

Davis, J.D. (1973) *The effectiveness of some sugars in stimulating licking behavior in the rat*. Physiol. Behav., 11, 39–45.

Davis, J.D. (1989) *The microstructure of ingestive behavior*. In Schneider, L.H., Cooper, S.J. and Halmi, K.A. (eds), The Psychobiology of Human Eating Disorders. Annals of the New York Academy of Sciences, New York, pp. 106–121.

Desimone, R., Olson, C. and **Erickson, R.** (1992) *The controlled water access program*. ILAR News, 34, 27–29.

Dudai, Y., Jan, Y.N., Byers, D. and **Quinn, W.G.** (1976) *Dunce, a mutant of* Drosophila *deficient in learning*. Proc. Natl Acad. Sci. USA, 73, 1684–1688.

Eylam, S. and **Spector, A.C.** (2002) *The effect of amiloride on operantly conditioned performance in a NaCl taste detection task and NaCl preference in C57BL/6J mice*. Behav. Neurosci., 116, 149–159.

Glendinning, J.I., Chaudhari, N. and **Kinnamon, S.C.** (2000) *Taste transduction and molecular biology*. In Finger, T., Silver, W.L. and Restrepo, D. (eds), The Neurobiology of Taste and Smell, 2nd edn. Wiley-Liss, New York, pp. 315–351.

Grill, H.J. and **Berridge, K.C.** (1985) *Taste reactivity as a measure of the neural control of palatability*. Prog. Psychobiol. Physiol. Psychol., 11, 1–61.

Grill, H.J., Spector, A.C., Schwartz, G.S., Kaplan, J.M. and **Flynn, F.W.** (1987) *Evaluating taste effects on ingestive behavior*. In Toates, F. and Rowland, N. (eds), Techniques in the Behavioral and Neural Sciences, Vol. 1: Feeding and Drinking. Elsevier, Amsterdam, pp. 151–188.

Halpern, B.P. (1977) *Functional anatomy of the tongue and mouth of mammals*. In Weijnen, J.A.W.M. and Mendelson, J. (eds), Drinking Behavior. Plenum Press, New York, pp. 1–92.

Heffner, H.E. and **Heffner, R.S.** (1995) *Conditioned avoidance*. In Klump,

G.M., Dooling, R.R., Fay, W.C. and Stebbins, W.C. (eds), Methods in Comparative Psychoacoustics. Birkhäuser, Basel, pp. 79–93.

Horowitz, G.P., Stephan, F.K., Smith, J.C. and **Whitney, G.** (1977) *Genetic and environmental variability in lick rates of mice*. Physiol. Behav., 19, 493–496.

Hughes, J.E., Amyx, H., Howard, J.L., Nanry, K.P. and **Pollard, G.T.** (1994) *Health effects of water restriction to motivate lever-pressing in rats*. Lab. Anim. Sci., 44, 135–140.

Inoue, M., McCaughey, S.A., Bachmanov, A.A. and **Beauchamp, G.K.** (2001) *Whole nerve chorda tympani responses to sweeteners in C57Bl/6ByJ and 129P3/J mice*. Chem. Senses, 26, 915–923.

Krimm, R.F., Nejad, M.S., Smith, J.C., Miller, I.J. and **Beidler, L.M.** (1987) *The effect of bilateral sectioning of the chorda tympani and the greater superficial petrosal nerves on the sweet taste in the rat*. Physiol. Behav., 41, 495–501.

Ninomiya, Y. and **Funakoshi, M.** (1993) *Genetic and neurobehavioral approaches to the taste receptor mechanism in mammals*. In Simon, S.A. and Roper, S.D. (eds), Mechanisms of Taste Transduction. CRC Press, Boca Raton, FL, pp. 253–272.

Nolan, P.M., Kapfhamer, D. and **Bucan, M.** (1997) *Random mutagenesis screen for dominant behavioral mutations in mice*. Methods: Compan. Methods Enzymol., 13, 379–395.

Nolan, P.M., Peters, J., Strivens, M., Rogers, D., Hagan, J., Spurr, N., Gray, I.C. *et al.* (2000) *A systematic, genome-wide, phenotype-driven mutagenesis programme for gene function studies in the mouse*. Nat. Genet., 25, 440–443.

Nowlis, G.H., Frank, M.E. and **Pfaffman, C.** (1980) *Specificity of acquired aversions to taste qualities in hamsters and rats*. J. Comp. Physiol. Psychol., 94, 932–942.

Rhinehart-Doty, J.A., Schumm, J., Smith, J.C. and **Smith, G.P.** (1994) *A non-taste cue of sucrose in short-term taste tests in rats*. Chem. Senses, 19, 425–431.

Sayah, D.M., Khan, A.H., Gasperoni, T.L. and **Smith, D.J.** (2000) *A genetic screen for novel behavioral mutations in mice*. Mol. Psychiatry, 5, 369–377.

Sayeed, O. and **Benzer, S.** (1996) *Behavioral genetics of thermosensation and hygrosensation in* Drosophila. Proc. Natl Acad. Sci. USA, 93, 6079–6084.

Smith, D.V. and **Davis B.J.** (2000) *Neural representation of taste*. In Finger, T., Silver, W.L. and Restrepo, D. (eds), The Neurobiology of Taste and Smell, 2nd edn. Wiley-Liss, New York, pp. 353–392.

Smith, D.V. and **Frank, M.E.** (1993) *Sensory coding by peripheral taste fibers*. In Simon, S.A. and Roper, S.D. (eds), Mechanisms of Taste Transduction. CRC Press, Boca Raton, FL, pp. 295–338.

Smith, J.C., Castonguay, T.W., Foster, D.W. and **Bloom, L.M.** (1980) *A detailed analysis of glucose and saccharin drinking in the rat*. Physiol. Behav., 24, 173–176.

Smith, J.C., Davis, J.D. and **O'Keefe, G.B.** (1992) *Lack of an order effect in brief contact taste tests with closely spaced test trials*. Physiol. Behav., 52, 1107–1111.

Spector, A.C. (2000) *Linking gustatory neurobiology to behavior in vertebrates*. Neurosci. Biobehav. Rev., 24, 391–416.

Spector, A.C. (2002) *Psychophysical evaluation of taste function in non-human mammals*. In Doty, R.L. (ed.), Handbook of Olfaction and Gustation, 2nd edn. Marcel Dekker, New York.

Spector, A.C., Grill, H.J. and **Norgren, R.** (1993) *Concentration-dependent licking of sucrose and sodium chloride in rats with parabrachial gustatory lesions*. Physiol. Behav., 53, 277–283.

Spector, A.C., Redman, R. and **Garcea, M.** (1996) *The consequences of gustatory nerve transection on taste-guided licking of sucrose and maltose in the rat*. Behav. Neurosci., 110, 1096–1109.

Spector, A.C., Klumpp, P.A. and **Kaplan, J.M.** (1998) *Analytical issues in the evaluation of food deprivation and sucrose concentration effects on the microstructure of licking behavior in the rat*. Behav. Neurosci., 112, 678–694.

St John, S.J., Garcea, M. and **Spector, A.C.** (1994) *Combined, but not single, gustatory nerve transection substantially alters taste-guided licking behavior to quinine in rats*. Behav. Neurosci., 108, 131–140.

Travers, J.B., DiNardo, L.A. and **Karimnamazi, H.** (1997) *Motor and premotor mechanisms of licking*., Neurosci. Biobehav. Rev., 21, 631–647.

Wahlsten, D. (2001) *Standardizing tests of mouse behavior: reasons, recommendations, and reality.* Physiol. Behav., 73, 695–704.

Weijnen, J.A.W.M. (1977) *The recording of licking behavior*. In Weijnen, J.A.W.M. and Mendelson, J. (eds), Drinking Behavior. Plenum Press, New York, pp. 93–114.

Whitney, G. and **Harder, D.B.** (1994) *Genetics of bitter perception in mice*. Physiol. Behav., 56, 1141–1147.

Wong, G.T., Gannon, K.S. and **Margolskee, R.F.** (1996) *Transduction of bitter and sweet taste by gustducin*. Nature, 381, 796–800.

Young, P.T. and **Trafton, C.L.** (1964) *Activity contour maps as related to preference in four gustatory stimulus areas of the rat*., J. Comp. Physiol. Psychol., 58, 68–75.

Accepted March 5, 2002