

Mouse Taste Preference Tests: Influence of Drinking Spout Position

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TORDOFF, M.G., AND BACHMANOV, A.A., Mouse taste preference tests: Influence of drinking spout position. *Unpublished*. The purpose of this study was to determine whether the results of two-bottle taste tests are influenced by cage layout, in particular, the position of the drinking spouts. To this end, we measured the 2 mM saccharin, 50 mM citric acid, 0.3 mM quinine hydrochloride, and 75 mM NaCl intakes and preferences of 129X1/SvJ (129) and C57BL/6J (B6) mice with various cage layouts. We found that the position of drinking spouts relative to the food (in a hopper or on the floor), the distance apart of the drinking spouts (2, 8, or 16 cm), or their relative orientation (horizontal or vertical) had no consistent effect on taste solution preferences. During tests with two bottles of water, the 129 (but not B6) mice demonstrated a left spout side preference when the spouts were close together (2 cm) but not when they were far apart (8 or 16 cm) or arranged vertically. These results suggest that drinking tube placement has little influence on the results of two-bottle tests, although placing the drinking tubes at least 8 cm apart reduces the spout side preferences displayed by some strains of mice.

Index terms: taste tests, saccharin, citric acid, quinine hydrochloride, NaCl, C57BL/6J, 129X1/SvJ, lateralization, side preference

It is common practice to assess the voluntary intake of taste and nutrient solutions by mice and rats using two-bottle tests. These involve a choice between a bottle of “taste solution” and bottle of water. Because the rodent is not forced to drink the taste solution to assuage hunger or thirst, this method provides a measure of voluntary or “spontaneous” consumption, and the ratio of taste solution intake to total intake is considered a measure of taste solution preference. “Taste solution” can be a misnomer in these experiments because solution intakes are influenced by non-taste factors, including the solution’s odor and trigeminal effects, the postingestive disposition of the ingested taste solution, and the subject’s metabolic state and previous experience. Nevertheless, for many compounds, the chemosensory properties of a solution are largely, if not completely, responsible for its preference, and

we therefore use “taste solution” and “taste tests” as convenient nomenclature.

As part of an effort to increase the efficiency of the two-bottle choice test for screening the offspring of mice with induced mutations (Tordoff and Bachmanov, 2001b), we examined the extent to which the position of drinking spouts influences taste solution preferences. Nearly 50 years ago, Gillespie and Lucas (1957) cautioned that “the size and shape of cage and the arrangement of self-selection dispensers may exert a strong influence on the apparent ‘preference’”. Despite this warning, little attention has been paid to spout position in previous research using two-bottle taste tests, and it is rare to find a methods section that provides details of cage layout. However, there is evidence that spout position can affect the results of two-bottle tests. Smith et al. (1976) reported that rats rapidly alternate between drinking saccharin solution from one spout and drinking glucose solution from another spout, and in a recent oral retrospective, Smith (2002) noted that this behavior was disrupted when a dividing wall was placed between the spouts. Ackroff et al (1993) found that rats given a choice between sucrose and Polycose drank roughly equal amounts of each when the spouts were placed 2.5 cm apart but mostly Polycose when the spouts were 11.5 cm apart. The authors of both studies concluded that with spouts close together, the rats could “orally mix” the taste solutions, which provided a more pleasant taste than did either solution alone.

Generally, intakes of both the taste solution and water are monitored for at least 48 h, and a recent paper shows that for some solutions the most sensitive results can be obtained with 96-h two-bottle tests (Tordoff and Bachmanov, 2002). There are at least three reasons why tests of at least 48-h are popular. First, intakes of rats (and larger animals) over a 48-h period can be measured accurately, with little concern about spillage or evaporation as confounding factors [see (Tordoff and Bachmanov, submitted) for discussion]. Second, the range of intakes typically observed in 48-h tests is large enough to observe differences between different groups of rats, treatments, or taste solutions. Third, 48-h tests allow controls for the

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confounding effects of side preferences: Some animals prefer one spout over the other [i.e., are left-“handed” (Bachmanov *et al.*, in press; Biddle and Eales, 1996; Gillespie and Lucas, 1957; 1958; Korn, 1960; Tordoff and Bachmanov, 2001a)] and so to counter this, the position of the drinking spouts is switched every 24 h. It is impractical to switch the spouts earlier because this fails to control for varying intakes during different periods of the circadian cycle.

We were particularly interested to find procedures that might reduce spout position preferences, which would allow us to use tests shorter than 48 h. In *Experiment 1*, we determined whether the distance between the two drinking spouts influenced taste or side preferences, and whether this was affected by the position of the food relative to the fluid bottles. In *Experiment 2*, we attempted to eliminate the influence of side preferences by arranging the drinking spouts vertically (i.e., one on top of the other).

In both experiments, we compared two strains of mice, the 129X1/SvJ (129) and C57BL/6J (B6) strain given various taste solutions to consume (2 mM saccharin, 50 mM citric acid, 0.3 mM quinine hydrochloride [QHCl], and 75 mM NaCl). The two mouse strains are used frequently in genetic studies, are among the first murine genomes to be sequenced, and are likely candidates for mutagenesis experiments. In a recent survey of 28 strains, a 129 sub-strain showed the strongest preference for the left drinking spout when given a choice between two drinking spouts; the B6 strain drank from left and right spouts about equally (Tordoff and Bachmanov, 2001a). There are very large differences between the B6 and 129 strains in saccharin preference, moderate differences in NaCl preference, small differences in citric acid preference, and little if any difference in QHCl preference [e.g. (Bachmanov *et al.*, 1996a; Bachmanov *et al.*, 1996b; Bachmanov *et al.*, 1998; Tordoff and Bachmanov, 2002; Tordoff *et al.*, 2002)]. This paper is one of a series in which we use the magnitude of the difference between the B6 and 129 strains to assess the effect of laboratory variables (i.e., spout number, test duration, diet, age) on the sensitivity of taste tests for genetic experiments (Tordoff and Bachmanov, 2001b; Tordoff and Bachmanov, 2002; submitted; Tordoff *et al.*, 2002).

METHODS

Subjects and maintenance

Both experiments used groups of 16 male C57BL/6J (B6) and 16 male 129X1/SvJ (129) mice that were purchased from The Jackson Laboratory (Bar Harbor, Maine) and were ~12 wk old at the start of testing. They were individually housed in plastic “tub” cages (26.5 cm x 17 cm x 12 cm) with a stainless steel grid lid, and wood shavings scattered on the floor [see (Bachmanov *et al.*, in press; Tordoff and Bachmanov, 2001b) for details; Fig. 1]. The vivarium was maintained at 23°C on a 12:12 h light/dark cycle with lights

off at 7 pm. The mice were fed pelleted Teklad 8604 chow (Harlan, Madison, WI), and had deionized water to drink.

Drinking tubes

During tests, the mice had access to two 25-ml drinking tubes. Throughout this paper we use the terms “drinking tubes” and “bottles” synonymously. We use “left” and “right” to refer to the position of drinking tubes from the viewpoint of a mouse facing the spouts (as in Fig. 1A). The tubes were fabricated from plastic pipettes, with stainless-steel drinking spouts and rubber stoppers. Each spout extended into the cage 25 mm and had a 3.175-mm diameter hole from which the mouse could lick fluids. Except for the “upper” tubes in *Experiment 2*, the end of the spouts rested ~2.5 cm above the plastic cage floor, although wood shavings covering the floor reduced the height the mice had to reach by a few millimeters. Specifics of construction of the drinking tubes are available in earlier papers (Bachmanov *et al.*, in press; Bachmanov *et al.*, 1996b) and in detail on the Monell Mouse Taste Phenotyping Project website (Tordoff and Bachmanov, 2001b). The only difference was that in earlier work we usually fastened to the cage lid a 60 mm x 15 mm metal “guard” sheet with two holes to accept the drinking spouts, spaced 2 cm apart. This protects the drinking tubes from being chewed by the mice. Instead here, each spout passed through a 2-cm diameter steel washer, which rested on the cage lid (*Experiment 1*), or the metal guard was turned to a vertical orientation (*Experiment 2*).

Taste solutions and general test procedure

In both experiments, the mice received several series of taste tests, which differed in the position of the drinking spouts. In each series, the following taste solutions were presented along with water: 2 mM saccharin, 50 mM citric acid, 300 µM QHCl, and 75 mM NaCl. All compounds were purchased from Sigma Chemical Corp (St. Louis, MO), and dissolved in deionized water (deionized water was also used in the drinking tubes containing water). Taste solutions were made freshly at the beginning of each experiment, and stored in 2 L plastic bottles until needed.

Each series began with a test involving water vs. water, and each test was 2 days long, so that the whole series lasted 10 days. In *Experiment 1*, the taste solution was always initially presented on the left and the water on the right. In *Experiment 2*, half the mice in each strain were tested with the taste solution initially presented on the left or bottom and the other half with it initially presented on the right or top. The position of the drinking tubes was reversed after the 24-h measurement of each 48-h test. Fluid intakes were measured daily in the middle of the light period, to the nearest 0.1 mL.

The order of taste tests was always the same (water, saccharin, citric acid, QHCl and NaCl) but each series was counterbalanced so that approximately equal numbers of

mice were tested in each spout position condition at the same time.

Each mouse's bedding was changed at the beginning of a series and after the test with 50 mM citric acid. If food was available on the cage floor, it was changed at the same time as bedding. If food was available from a hopper, it was refilled when needed. Body weights were measured at the beginning and end of each experiment.

Experiment designs and specific test procedures

Experiment 1. Influence of inter-spout distance and food position on taste solution preferences This experiment involved an incomplete 3 x 2 design, with each mouse being tested five times. The factor involving three levels was the distance apart of the drinking spouts (2, 8, or 16 cm). The factor involving two levels was the position of the food in the cage (in the lid hopper or on the floor). The design was incomplete because it was not possible to test the mice with 16 cm between the drinking spouts when the food was in the cage lid hopper.

For the conditions with drinking spouts 2 cm apart, the drinking tubes were arranged so they almost touched each other (Fig. 1A). For conditions with the drinking spouts 8 cm apart, the drinking tubes were arranged with one ~0.5 cm from the wall of the cage and the other ~0.5 cm from the metal divider of the food hopper. For the condition with the drinking spouts 16 cm apart, the drinking tubes were arranged to be ~0.5 cm from each wall of the cage (Fig. 1B). For conditions with food in the hopper, no special procedures were followed. For conditions with food on the floor, between 6 – 10 pellets (each ~25 x 16 x 10 mm) were scattered on the cage floor.

Experiment 2. Comparison of tests using bottles arranged vertically on taste solution preferences. Several lines of evidence, including the results of *Experiment 1*, show that some mice have strong spout side preferences when drinking tubes are presented side-by-side [e.g., (Bachmanov et al., in press)]. We argued that these side preferences could be eliminated by presenting the drinking spouts in a vertical orientation, although it was possible that this procedure might introduce other biases related to spout height. In this experiment, each mouse was given two series of two-bottle tests. One series involved the standard choice tests, with the two drinking tubes presented 2-cm apart and both spout tips at ~2.5 cm above the cage floor. The other series involved presenting the drinking tubes such that one spout was 2 cm directly above the other. In this series, the lower drinking tube rested on the cage lid, in a similar manner to the tubes in the standard, left-right condition (spout tip ~2.5 cm above the floor). The upper tube was parallel to and above the lower one (spout tip ~4.5 cm above the floor). It was supported by a metal chew guard at one end (see Drinking tubes) and a Plexiglas block at the other (Fig. 1C).

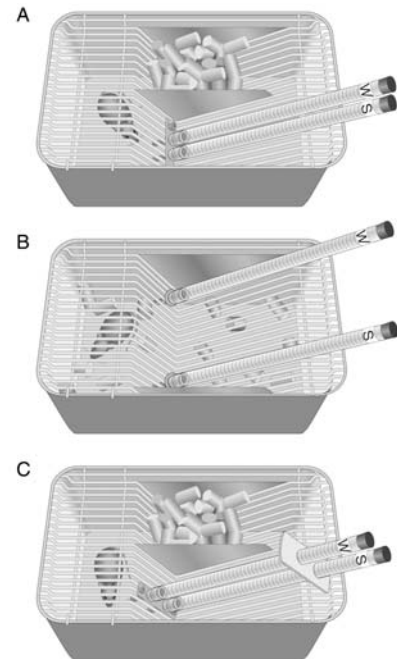


Fig.1. Schematics of some of the cage layouts used in Experiments 1 and 2. A. Standard cage and two-bottle test with drinking spouts 2-cm apart (used in *Experiment 2*; in *Experiment 1*, the layout was identical except that the rubber stoppers were protected from bites by steel washers rather than the metal strip shown here). B. Drinking spouts 16 cm apart, food on floor layout used in *Experiment 1*. C. Layout of tubes for “up-down” or vertical orientation used in *Experiment 2*.

Statistical analyses

At the start of testing, there were significant differences between the strains in body weight in *Experiment 2* ($B6 = 24 \pm 0.3$ g; $129 = 26 \pm 0.4$ g, $t(30) = 3.73$, $p < 0.05$) but not in *Experiment 1* ($B6 = 24 \pm 0.3$ g, $129 = 25 \pm 0.5$ g; NS). Despite the statistically significant difference, the actual differences between the means were small. We thus concluded that there was no need to consider the effect of body weight on solution intake [see (Bachmanov et al., in press; Bachmanov et al., 1998) for discussion]. This considerably simplified subsequent analyses.

All hypothesis testing used a criterion of $p < 0.05$ for statistical significance, but exact probabilities are given in the text. Values given in the text and tables are means \pm SEMs.

Spout side preferences. Spout side preferences were derived from tests in which water was presented in both drinking tubes. We determined the volume of water ingested by each mouse from the left (or top) and right (or bottom) tubes, and its side preference (intake from left or top tube/total fluid intake). The existence of a significant side preference for a given strain and condition was inferred by comparing the mean preference with indifference (i.e., 50%) using one-sample t -tests. Differences in side

Table 1. Influence of inter-spout distance and food position on water intake and spout side preferences of B6 and 129 mice given two bottles of water to drink.

Measure of intake	Strain	Food in hopper		Food on floor		
		2 cm ¹	8 cm	2 cm	8 cm	16 cm
Left spout, mL	129	3.2 ± 0.3	2.5 ± 0.3	3.3 ± 0.2	2.9 ± 0.3	2.6 ± 0.4
	B6	2.8 ± 0.2	2.9 ± 0.2	3.2 ± 0.1	3.0 ± 0.2	2.9 ± 0.3
Right spout, mL	129	1.8 ± 0.2*	2.9 ± 0.4	1.8 ± 0.1*	2.3 ± 0.3	2.9 ± 0.3
	B6	2.9 ± 0.2	2.9 ± 0.2	2.4 ± 0.2	2.3 ± 0.2	3.0 ± 0.2
Total intake, mL	129	4.9 ± 0.2	5.4 ± 0.3	5.1 ± 0.1	5.2 ± 0.3	5.5 ± 0.2
	B6	5.7 ± 0.2	5.8 ± 0.2	5.5 ± 0.2	5.3 ± 0.3	5.9 ± 0.2
Left preference, %	129	65 ± 5*†	46 ± 6	65 ± 3*†	54 ± 5	48 ± 6
	B6	49 ± 2	49 ± 4	57 ± 2†	54 ± 3	49 ± 4

Values in body of table are means ± SEs. *p < 0.05 relative to B6 strain, †p < 0.05 relative to indifference (50% preference). ¹Distance apart of drinking spouts in centimeters

preference scores between the strains were analyzed by ANOVA.

We also analyzed intakes from the left and right drinking spouts on each of the two days of each test involving taste solutions. The general finding of these analyses was that taste solution preferences were usually higher on days when this was presented on a mouse's more-favored side than when it was presented on its less-favored side (determined from the water vs. water test). However, the results were complex and not as easy to interpret as those seen with water in both drinking tubes, so they will not be presented here.

Strain differences and effects of cage layout in taste tests. For tests involving taste solutions, fluid intakes from each drinking tube for each mouse were averaged across the two days of the test, and collated according to the taste solution and test condition. For each mouse on each test, solution preference ratios were calculated based on the formula: preference (%) = taste solution intake / (taste solution intake + water intake) × 100. Subsequent analyses were conducted in parallel, using taste solution intakes, water intakes, total fluid intakes, and taste solution preferences as dependent variables.

For Experiment 1, mixed design ANOVAs were used, with factors of Strain (129 or B6) and Condition. Additional planned comparisons involving the effects of food position (in the lid hopper or on the floor) and inter-spout distances (2, 8 and 16 cm) were also conducted. For Experiment 2, the ANOVAs involved factors of Strain (19 or B6), Spout Orientation (horizontal or vertical), and Taste Solution Spout Position (initially on the left or top vs. initially on the right or bottom). However, there were no significant effects or interactions involving the third factor, so for simplicity, the data were re-analyzed without it.

Influence of cage layout on taste test sensitivity. We have developed a method to allow cross-experiment comparisons of the sensitivity of taste tests, based on the assumption that this is reflected by the difference between B6 and 129 strains (Tordoff and Bachmanov, in press; Tordoff et al., 2002). Here, we compared the variability of the response in solution intakes and preference scores of the two strains observed in each of the seven spout layout conditions in Experiments 1 and 2. The method takes into account both the magnitude of the difference between strain means and the within-strain variability. To do this, we normalized all data from each test to the B6 group mean. That is, for each test, we calculated z scores, based on the mean of the B6 group and the average standard deviations of the B6 and 129 groups. The normalized values of the 129 mice were collated for each cage layout condition and then compared using one-way analyses of variance [see (Tordoff and Bachmanov, 2002) for additional justification]. To conserve space, only the analyses of taste preference scores are presented here.

RESULTS

Experiment 1. Influence of inter-spout distance and food position on taste solution preferences

Side preferences during tests with two bottles of water. With two drinking tubes providing water, the 129 mice showed significant preferences for the left spout if the drinking spouts were positioned 2-cm apart, but not if they were positioned 8- or 16-cm apart [Table 1; 1-sample t-tests relative to 50% preference, 129 mice, spouts 2-cm apart, food in hopper, $t(15) = 3.10$, $p = 0.004$; 129 mice, spouts 2-cm apart, food on floor, $t(15) = 5.13$, $p < 0.0001$]. The B6 mice also showed a significant preference for the left spout if the spouts were 2 cm apart and the food was on the cage floor, $t(15) = 3.82$, $p = 0.008$. However, the B6 mice did not show side preferences in other conditions, and the left spout

Table 2. Results of ANOVAs (F-Values) comparing B6 and 129 tested under five conditions in which the inter-spout distance and position of the food were manipulated (Experiment 1)

Source of variance and dependent variable	2 mM saccharin	50 mM citric acid	0.3 mM QHCl	75 mM NaCl
Effect of strain (df = 1 and 30)				
Water intake	177.9***	0.26	0.38	0.20
Solution intake	293.8***	0.42	0.23	2.12
Total fluid intake	90.2***	0.44	0.52	9.10**
Solution preference	275.3***	0.70	0.09	7.40*
Effect of cage layout¹ (df = 4 and 120)				
Water intake	1.21	0.79	2.81*	2.21
Solution intake	0.75	2.07	1.26	2.59*
Total fluid intake	1.40	0.93	2.02	3.73*
Solution preference	0.48	2.56*	1.99	1.44
Strain x condition interaction (df = 4 and 120)				
Water intake	1.90	0.65	1.19	0.45
Solution intake	0.67	1.44	0.63	2.15
Total fluid intake	0.62	0.24	0.95	1.18
Solution preference	0.95	1.75	0.74	1.23

*P<0.05; **p<0.001, ***P<0.001. ¹The five cage layouts tested were (a) spouts 2-cm apart, food in hopper, (b) spouts 8-cm apart, food in hopper, (c) spouts 16-cm apart, food in hopper, (d) spouts 2-cm apart, food on floor, and (e) spouts 8-cm apart, food on floor.

preference shown by the 129 mice was significantly greater than that of the B6 mice [strain x spout distance interaction, $F(1,30) = 4.48, p = 0.04$]. The strain difference was due largely to lower water intakes of 129 than B6 mice from the right spout (Table 1). There were no differences in intake from the left drinking spouts or in total water intake between the strains.

Tests with taste solutions. There was no evidence that the distance between drinking spouts or food position had a differential effect on the response of the two strains of mice to any of the taste solutions (all strain x test condition interactions were nonsignificant, Table 2; to conserve space, we present only preference scores (Fig. 2)]. In all test conditions, B6 mice had significantly higher preferences for saccharin than did 129 mice. This was due to significantly higher intakes of saccharin (and total fluid), and significantly lower intakes of water (Table 2). B6 mice also had significantly higher preferences for NaCl than did 129 mice in all tests combined (Fig. 2; Table 2). There were no differences between the strains in preferences or intakes of citric acid or QHCl.

For both strains combined, cage layout significantly influenced citric acid preference, water intake during tests with QHCl available, NaCl intake, and total fluid intake when NaCl was available (Table 2). However, these effects were small (all p 's<0.05 but >0.01) and inconsistent: The effect on citric acid preference was due to significantly higher preferences in the spouts 16-cm apart condition than in the other conditions (except the spouts 8-cm apart, food in hopper condition). The effect on water intake when QHCl was available was due to significantly higher water intakes in the spouts 2-cm apart, food in hopper condition than in the conditions

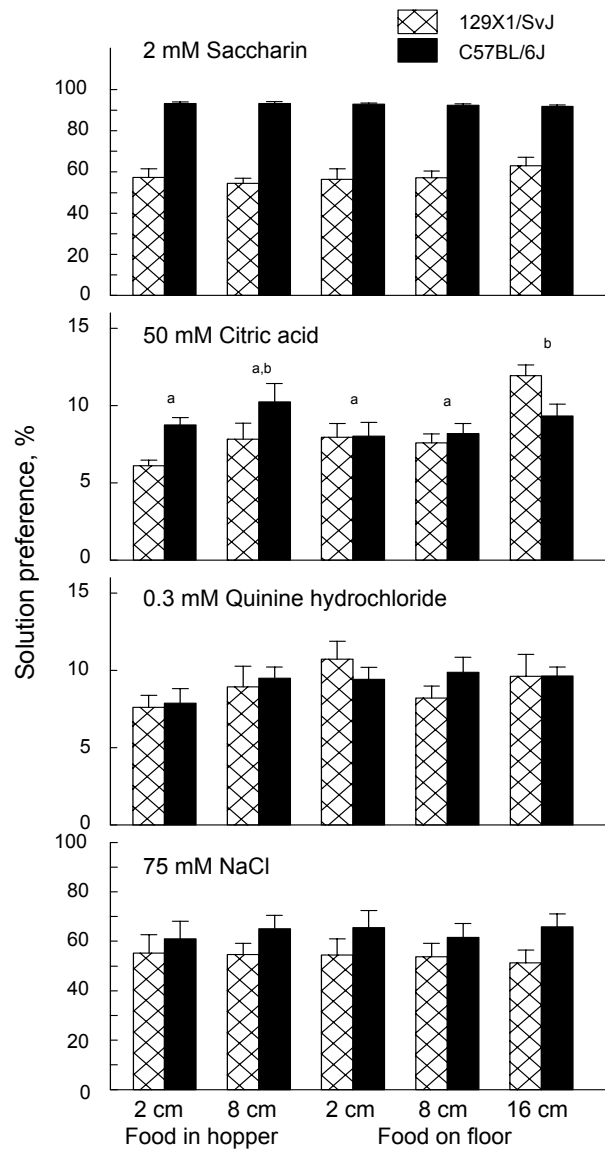


Fig 2 . Preferences of 129X1/SvJ (129) and C57BL/6J (B6) mice for four taste solutions during 48-h tests with the distance between drinking spouts and food position manipulated. *p<0.05, 129 vs. B6 strains. B6 mice had significantly higher preferences for saccharin and NaCl than did 129 mice over all conditions combined. Letters above columns in citric acid panel show differences between the conditions irrespective of strain. Columns with the same letter did not differ significantly from each other (p<0.05). There were no significant differences between conditions for any of the other taste solutions.

with the spouts 2-cm or 8 cm apart, food on floor conditions, and between the spouts 8-cm apart, food in hopper condition and the three conditions with food on the floor. The differences in NaCl intake and total fluid intake when NaCl was available were due to mice in the spouts 8-cm apart, food in hopper drinking more NaCl than did mice in the other four conditions.

Experiment 2. Influence of horizontal or vertical spout arrangement on taste solution preferences

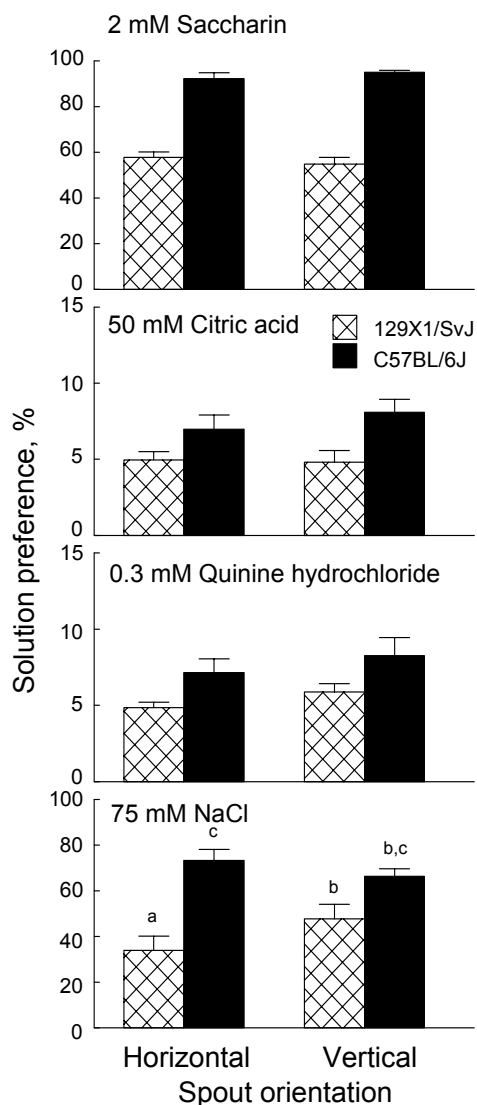


Fig 3. Influence of spout orientation on taste solution preference of 129X1/SvJ and C57BL/6J mice. For all four taste solutions, B6 mice had higher preference scores than did 129 mice. Spout orientation had no effect on preferences for saccharin, citric acid or QHCl. However, there was an interaction between strain and spout orientation for NaCl preference scores. Columns with the same letter did not differ significantly from each other ($p < 0.05$).

Side preferences during tests with two bottles of water. In close agreement with the results of Experiment 1, when two drinking tubes containing water were provided side-by-side, 129 mice preferred to drink from the left spout whereas B6 mice drank similar amounts from each spout (Table 3; strain \times side interaction, $F(1,30) = 4.87$, $p = 0.035$; 129 preference, $t(15) = 3.43$, $p = 0.004$). When the drinking tubes were provided vertically (one above the other), both strains of mice drank similar amounts from each spout (Table 3).

Tests with taste solutions. B6 mice had significantly higher preference scores for all four taste solutions than did 129 mice (Table 4; Fig. 3). In each case, this was because the B6 mice drank significantly more of the taste solution than did the 129 mice. The B6 mice also drank significantly more water than did the 129 mice during tests with citric

Table 3. Intake and spout side preference of mice given two drinking tubes containing water arranged either horizontally or vertically (Experiment 2)

Spout arrangement and source of water	129	B6
Horizontal		
Left spout, mL	3.1 \pm 0.3	2.7 \pm 0.2
Right spout, mL	1.4 \pm 0.2*	2.3 \pm 0.2
Total intake, mL	4.5 \pm 0.2	5.0 \pm 0.2
Preference, % ¹	68 \pm 5*†	54 \pm 3
Vertical		
Top spout, mL	2.6 \pm 0.4	2.4 \pm 0.2
Bottom spout, mL	1.9 \pm 0.3	2.4 \pm 0.2
Total intake, mL	4.5 \pm 0.2	4.8 \pm 0.2
Preference, % ¹	45 \pm 8	50 \pm 4

* $p < 0.05$ relative to B6 strain, † $p < 0.05$ relative to indifference (50% preference). Values are means \pm SEM. ¹Refers to preference for left or top spout relative to total intake.

acid and QHCl, but significantly less water during tests with saccharin and NaCl. Total fluid intake of B6 mice was always significantly higher than total fluid intake of 129 mice (Table 4).

Mice of both strains had higher total fluid intakes when their drinking spouts were arranged vertically rather than horizontally. This was a significant difference for tests involving saccharin, citric acid, and NaCl, and bordered on significance for QHCl ($p < 0.06$). The higher total fluid intakes appeared to be due to higher intakes of both taste solution and water because in no case did spout orientation significantly influence taste solution intake or preference, and in only one case (for citric acid) did it significantly influence water intake.

B6 and 129 mice responded differently in tests with horizontal vs. vertical spout orientation when saccharin and NaCl were the taste solutions. The B6 strain drank significantly more saccharin when spouts were arranged vertically than horizontally (8.4 ± 0.4 mL/d vs. 7.5 ± 0.4

Table 4. Results of ANOVAs (F-values) comparing B6 and 129 mice tested with drinking spouts arranged horizontally or vertically (Experiment 2)

Source of variance and dependent variable	2 mM saccharin	50 mM citric acid	0.3 mM QHCl	75 mM NaCl
Effect of strain (df = 1 and 30)				
Water intake	98.0****	4.57*	7.70**	9.66***
Solution intake	190.3****	10.4***	13.7***	28.1****
Total fluid intake	116.5****	9.86**	13.0***	19.8***
Solution preference	235.0****	8.24**	9.58***	19.2***
Effect of spout orientation (df = 1 and 30)				
Water intake	0.02	7.62**	1.39	0.04
Solution intake	3.48	1.39	2.15	2.45
Total fluid intake	5.19*	8.55**	4.02	6.90*
Solution preference	0.00	0.63	1.58	1.00
Strain \times spout orientation interaction (df = 1 and 30)				
Water intake	1.26	2.65	1.14	8.42**
Solution intake	6.45*	0.64	0.29	6.84*
Total fluid intake	5.09*	1.15	0.45	0.37
Solution preference	1.73	1.05	0.00	8.80**

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$; **** $p < 0.00001$

mL/d) whereas intakes for the 129 strain were similar during the two test types (2.6 ± 0.2 mL/d vs. 2.4 ± 0.1 mL/d, respectively). This significant interaction was also reflected in total fluid intakes but not in saccharin preference scores (Table 4). In contrast, the 129 strain drank significantly more NaCl when spouts were arranged vertically than horizontally (2.5 ± 0.4 mL/d vs. 1.6 ± 0.3 mL/d, respectively) whereas intakes for the B6 strain were similar during the two test types (4.1 ± 0.3 mL/d vs. 4.3 ± 0.3 mL/d, respectively). A reciprocal pattern was seen with water intakes (B6 horizontal = 1.5 ± 0.3 mL/d, B6 vertical = 2.1 ± 0.2 mL/d, 129 horizontal = 3.1 ± 0.3 mL/d, 129 vertical = 2.5 ± 0.3 mL/d). This led to highly significant strain \times spout orientation interaction affecting NaCl preference scores (Fig. 3).

Comparison of test sensitivities

Significant effects of spout layout on taste test sensitivity were found for all taste solutions [saccharin, $F(6,105) = 7.71$, $p < 0.0001$; citric acid, $F(6,105) = 6.20$, $p < 0.0001$; QHCl, $F(6,105) = 2.78$, $p = 0.015$; NaCl, $F(6,105) = 5.61$, $p < 0.0001$]. Differences between individual tests are shown in Table 5. Although there were several significant differences in the effectiveness of tests for specific taste solutions, no spout layout was consistently better than the others.

DISCUSSION

Studies showing that rats orally mix solutions from drinking spouts presented close together but not far apart (Ackroff *et al.*, 1993; Smith *et al.*, 1976) raise the possibility

that spout position could affect the response of mice in two-bottle tests involving a choice between water and a taste solution. However, the results found here suggest this is not the case, at least for mice given prototypical sweet, sour, bitter, or salty compounds. Drinking spout position and cage layout did not have concerted effects on strain mean intakes or preferences, or on the tests' sensitivity to discriminate between strains.

There were occasional significant differences between intakes or preferences related to cage layout, and several significant differences related to test sensitivity (Table 5). Some of these differences were probably due to Type I statistical errors. However, most appear to have been due to an uncontrolled difference between the mice used in *Experiment 1* and *2*. There were two findings that lead us to this conclusion; First, there were significant differences in body weight between the B6 and 129 strains in *Experiment 2* but not *1*. Second, the "2-cm apart, food in hopper condition" of *Experiment 1* and "horizontal spout position" condition of *Experiment 2* were virtually identical but did not produce identical results. The only procedural difference was the use of steel washers or a small metal plate to protect the drinking tubes from bites. However, the sensitivity of the test to distinguish between NaCl preferences of B6 and 129 mice was significantly greater in *Experiment 2* than *1*. It is conceivable that carry-over effects from the results of other tests in each experiment influenced the results, although we have been unable to demonstrate any carry-over effects in controlled experiments specifically designed to investigate them (unpublished results). Instead, we strongly suspect that

Table 5. Influence of drinking spout layout on the variability of preference scores: Deviation of B6 and 129 mice from the mean value of the B6 strain¹

Cage layout	strain	2 mM saccharin	50 mM citric acid	0.3 mM QHCl	75 mM NaCl
Spouts 2-cm apart, food in hopper	B6	0.00 ± 0.07	0.00 ± 0.28	0.00 ± 0.27	0.00 ± 0.25
	129	3.68 ± 0.43^a	0.55 ± 0.22^c	$0.08 \pm 0.23^{a,b}$	0.20 ± 0.25^a
Spouts 8-cm apart, food in hopper	B6	0.00 ± 0.13	0.00 ± 0.26	0.00 ± 0.18	0.00 ± 0.27
	129	5.50 ± 0.37^b	$0.54 \pm 0.24^{a,b,c}$	$0.14 \pm 0.32^{a,b}$	0.09 ± 0.23^a
Spouts 2-cm apart, food on floor	B6	0.00 ± 0.05	0.00 ± 0.25	0.00 ± 0.20	0.00 ± 0.26
	129	3.16 ± 0.45^a	$0.01 \pm 0.25^{a,b}$	-0.34 ± 0.30^a	0.04 ± 0.24^a
Spouts 8-cm apart, food on floor	B6	0.00 ± 0.09	0.00 ± 0.26	0.00 ± 0.28	0.00 ± 0.25
	129	$4.23 \pm 0.41^{a,b}$	$0.25 \pm 0.24^{a,b}$	$0.48 \pm 0.22^{a,b}$	0.36 ± 0.25^a
Spouts 16-cm apart, food on floor	B6	0.00 ± 0.08	0.00 ± 0.11	0.00 ± 0.14	0.00 ± 0.25
	129	2.96 ± 0.42^a	-0.38 ± 0.39^a	$0.01 \pm 0.36^{a,b}$	$0.70 \pm 0.25^{a,b}$
Spouts arranged horizontally	B6	0.00 ± 0.26	0.00 ± 0.32	0.00 ± 0.36	0.00 ± 0.21
	129	3.43 ± 0.42^a	$0.68 \pm 0.18^{a,b,c}$	0.90 ± 0.14^b	1.79 ± 0.29^b
Spouts arranged vertically	B6	0.00 ± 0.11	0.00 ± 0.26	0.00 ± 0.34	0.00 ± 0.17
	129	5.62 ± 0.39^b	1.02 ± 0.24^c	$0.69 \pm 0.16^{a,b}$	$0.98 \pm 0.33^{a,b}$

¹Values in the body of the table show mean \pm SEM of z scores, based on the mean of the B6 group and the average standard deviations of the B6 and 129 groups. Thus, for example, in the "Spouts 2-cm apart, food in hopper" condition with 2 mM saccharin, the mean of the 129 mice differed from the mean of B6 mice by 3.68 standard deviations. Negative values show that 129 mean was higher than B6 mean. A significant difference in the analysis of variance signifies that there were differences among the tests in their ability to distinguish B6 from 129 mice. Values with the same superscript did not differ significantly from each other ($P < 0.05$, Tukey's test). Note that, despite the different names, the "spouts 2-cm apart, food in hopper", and "spouts arranged horizontally" are essentially identical spout layouts.

some aspect of husbandry or shipping that occurs before the mice arrive in our facility can produce long-lasting changes in their taste solution ingestion. Crabbe et al (1999) have emphasized the difficulty of producing replicable results even when heroic measures are made to standardize animal husbandry and experimental procedures across laboratories. Our results suggest that the same problems may occur even within the same laboratory using mice obtained at different times.

Spout side preferences. Spout side preference is a sufficiently well-known phenomenon that nearly all investigators control for it by switching spout positions during tests, but there have been no studies of its cause. It is likely, although not proved, that spout preference is a genetic predisposition. Using standard two-bottle, water vs. water choice conditions (i.e., with spouts 2-cm apart), we tested 28 inbred mouse strains and found 13 significantly favored the left spout and 15 showed indifference; none significantly favored the right spout (Bachmanov et al., in press; Tordoff and Bachmanov, 2001a). Spout side preference is apparently unrelated to mouse handedness, as determined by monitoring the paw used to obtain a food pellet at the bottom of a narrow tube (Bachmanov et al., in press; Biddle and Eales, 1996).

Confirming earlier results, we found here that B6 mice were largely indifferent to spout position (Bachmanov et al., in press; Tordoff and Bachmanov, 2001a; Tordoff and Bachmanov, submitted). They showed a significant preference for the left spout in only one of the six left-right spout layout conditions tested. We also found that 129X1/SvJ mice, like the 129P3/J strain tested earlier, strongly favored the left drinking spout when the spouts were close together. However, the 129X1/SvJ's side preference was eliminated by positioning the spouts 8 or 16 cm apart. The mice also did not show a preference related to the height the drinking spouts were presented; they drank similar amounts of taste solution from spouts located ~2.5 and ~4.5 cm above the floor.

We originally thought that the mice favored one or the other spout because of their cage layout. Under the test conditions we have used most frequently to date (Fig 1A), the left spout is closest to the food hopper and we thought that the mouse might seek the closest source of water after eating food, or simply consider the left spout the "safest" because the space underneath the food hopper provides shelter from potential predator-technicians. However, this did not appear to be the case because the 129 strain's side preference was present even if the food hopper was removed and food was scattered randomly on the cage floor. It also did not appear to be related to the distance of the spouts from the cage wall, because there were no side preferences displayed when the spouts were 8 cm apart, even though the left spout was much further from a cage wall than was the right one. We conclude that spout preference is not a spatial preference relative to the cage walls, food, or nesting place but more likely reflects a

genetically determined form of lateralization that is distinct from lateralization related to paw usage.

Recommendations for future research. This work is part of a project to evaluate methods to phenotype large numbers of mice for genetic studies. The two-bottle choice method has already been used extensively with many hundreds of mice to characterize quantitative trait loci [e.g., (Bachmanov et al., 2001)], and will likely be used to test many thousands of mice with induced mutations (The Jackson Laboratory, 2001). These projects occur in different institutions and there is no standardization of testing procedures, including cage layout or drinking spout position. The present results show that the taste solution intakes and preferences of 129 and B6 mice are largely unaffected by these factors. We conclude from this that neither cage layout nor drinking spout position is likely to be a critical contributor to experiment variance, although we stress that this is based on the results from only two strains of mouse and one type of cage. Investigators using other strains or cage types would be wise to conduct their own evaluations.

One area where cage layout influenced the results was in the expression of spout side preferences. Specifically, we found that 129 mice drank consistently more from the left spout when the spouts were presented side-by-side but not when they were separated by 8 or 16 cm. Tests in which spout side preferences were absent were not noticeably more sensitive. Nevertheless, it seems a good strategy to avoid potential problems related to spout side preference by positioning drinking tubes at least 8 cm apart if this is feasible. Without the confound of side preferences, it may be possible to use tests shorter than 48 h and still maintain reasonable test sensitivity.

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References

- Ackroff, K., Manza, L. and Sclafani, A. (1993). *The rat's preference for sucrose, Polycose and their mixtures*. *Appetite*, 21, 69-80.
- Bachmanov, A.A., Li, X., Reed, D.R., Ohmen, J.D., Li, S., Chen, Z., Tordoff, M.G., de Jong, P.J., Wu, C., West, D.B., Chatterjee, A., Ross, D.A. and Beauchamp, G.K. (2001). *Positional cloning of the mouse saccharin preference (Sac) locus*. *Chem Senses*, 26, 925-933.
- Bachmanov, A.A., Reed, D.R., Beauchamp, G.K. and Tordoff, M.G. (in press). Body weight, food intake, water intake, and drinking spout side preference of 28 mouse strains. *Behav. Genetics*.
- Bachmanov, A.A., Reed, D.R., Tordoff, M.G., Price, R.A. and Beauchamp, G.K. (1996a). Intake of ethanol, sodium chloride, sucrose, citric acid, and quinine hydrochloride solutions by mice: a genetic analysis. *Behav. Genetics*, 26, 563-573.
- Bachmanov, A.A., Tordoff, M.G. and Beauchamp, G.K. (1996b). *Ethanol consumption and taste preferences in C57BL/6ByJ and 129/J mice*. *Alcohol Clin. Exp. Res.*, 20, 201-206.

- Bachmanov, A.A., Tordoff, M.G. and Beauchamp, G.K. (1998).** *Voluntary sodium chloride consumption by mice: differences among five inbred strains.* *Behav. Genetics*, **28**, 117-124.
- Biddle, F.G. and Eales, B.A. (1996).** The degree of lateralization of paw usage (handedness) in the mouse is defined by three major phenotypes. *Behav Genet*, **26**, 391-406.
- Crabbe, J.C., Wahlsten, D. and Dudek, B.C. (1999).** *Genetics of mouse behavior: interactions with laboratory environment.* *Science*, **284**, 1670-1672.
- Gillespie, R.J.G. and Lucas, C.C. (1957).** An unsuspected factor which influences consumption of alcohol by rats. *Nature*, **180**, 1292-1293.
- Gillespie, R.J.G. and Lucas, C.C. (1958).** *An unexpected factor affecting the alcohol intake of rats.* *Canadian Journal of Biochemistry and Physiology*, **36**, 37-44.
- Korn, S.J. (1960).** The relationship between individual differences in responsivity of rats to stress and intake of alcohol. *Quart. J. Studies Alcohol*, **21**, 605-637.
- Smith, J.C.** Mentors and colleagues: the evolving of science (Johnson & Johnson Career Award for Research in Ingestive Behavior). 2002; Santa Cruz, CA.
- Smith, J.C., Williams, D.P. and Short Jue, S. (1976).** *Rapid oral mixing of glucose and saccharin by rats.* *Science*, **191**, 304-305.
- The Jackson Laboratory (2001).** *Neuroscience mutagenesis facility.* <http://www.jax.org/nmf/documents/overview.html>
- Tordoff, M.G. and Bachmanov, A.A. (2001a).** *Food intakes, water intakes, and spout side preferences.* <http://aretha.jax.org/pub-cgi/phenome/mpdcgi?rtn=studies/details&id=63>
- Tordoff, M.G. and Bachmanov, A.A. (2001b).** *Monell mouse taste phenotyping project.* www.monell.org/MMTPP
- Tordoff, M.G. and Bachmanov, A.A. (2002a).** Influence of test duration on the sensitivity of the two-bottle choice test. *Chem Senses*, **27**, 521-529.
- Tordoff, M.G. and Bachmanov, A.A. (2002b).** Influence of test duration on the sensitivity of the two-bottle choice test. *Chem Senses*, **27**, 759-768.
- Tordoff, M.G. and Bachmanov, A.A. (2003).** Mouse taste preference tests: Why only two bottles? *Chem Senses*. **28**,:315-24.
- Tordoff, M.G., Pilchak, D.M., Williams, J.A., McDaniel, A.H. and Bachmanov, A.A. (2002).** The maintenance diets of C57BL/6J and 129X1/SvJ mice influence their taste solution preferences: Implications for large-scale phenotyping projects. *J. Nutr.*, **132**, 2288-2297.